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Huberman

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(54) **COMPOSITIONS AND METHODS FOR
TREATING VIRAL DISEASES**

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A61K 31/437 (2006.01)

A61K 31/44 (2006.01)

C07D 471/04 (2006.01)

(52) **U.S. Cl.**

CPC **A61K 31/437** (2013.01); **A61K 31/44**
(2013.01)

(58) **Field of Classification Search**

CPC A61K 31/44; A61K 31/437; C07D 471/04

USPC 514/300; 546/113

See application file for complete search history.

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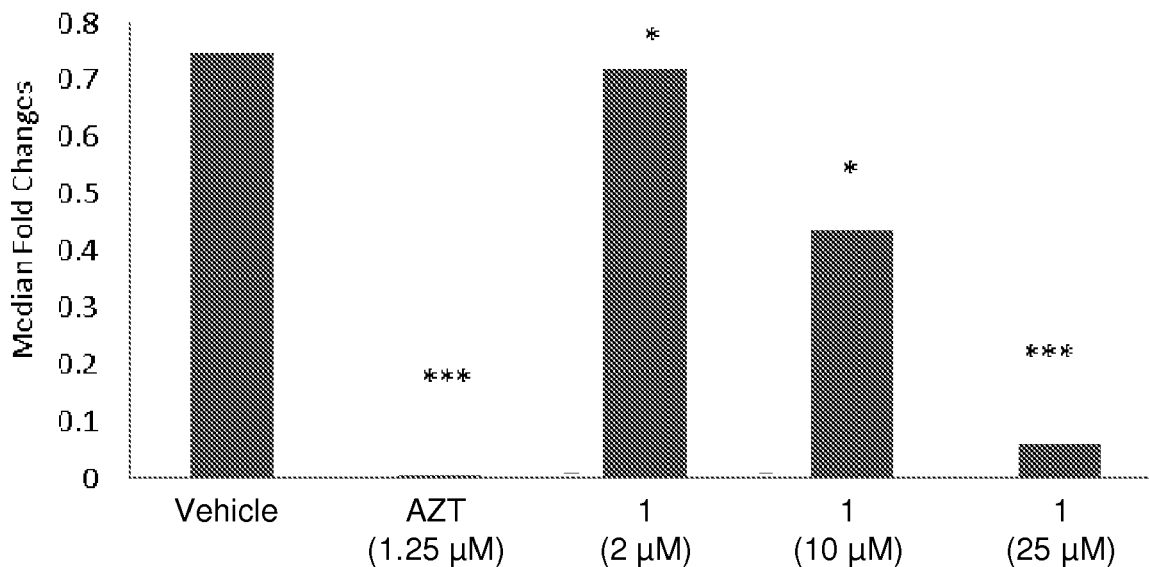
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ABSTRACT

Substituted perhydro pyrrolopyridines and methods for their use in the treatment of HIV infections, AIDS, and AIDS-related diseases, and in the treatment of BVDV infections are described herein. Also, pharmaceutical compositions comprising the substituted perhydro pyrrolopyridines are useful for the treatment of HIV infections, AIDS, and AIDS-related diseases. The compositions include one or more carriers, diluents, or excipients, or a combination thereof.

24 Claims, 10 Drawing Sheets



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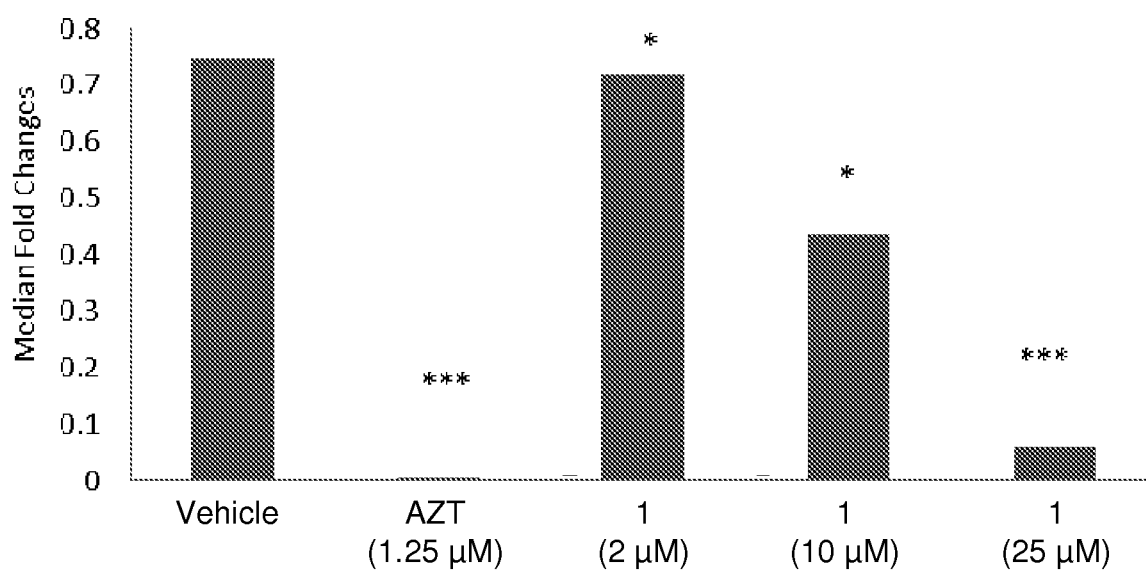
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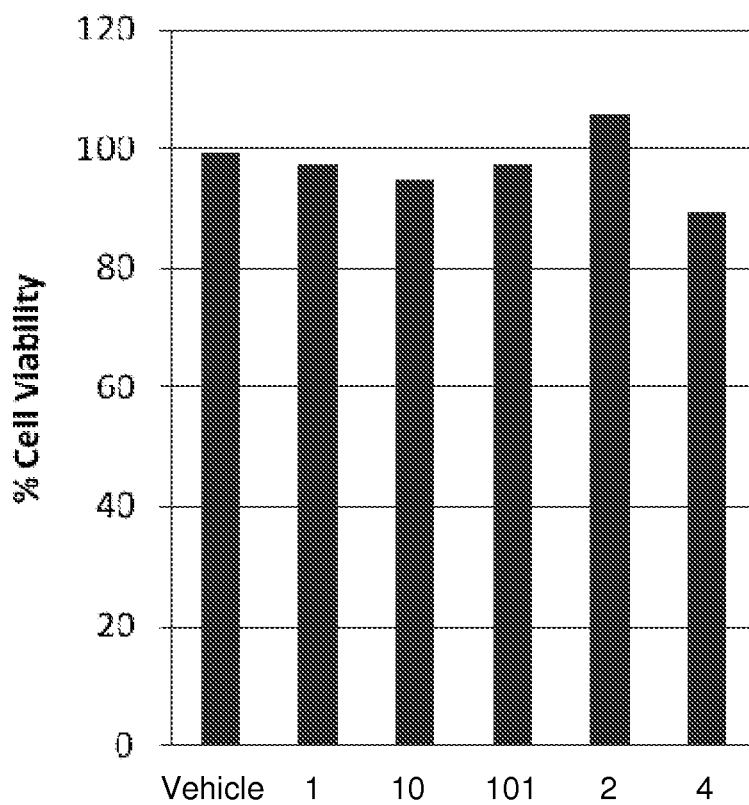
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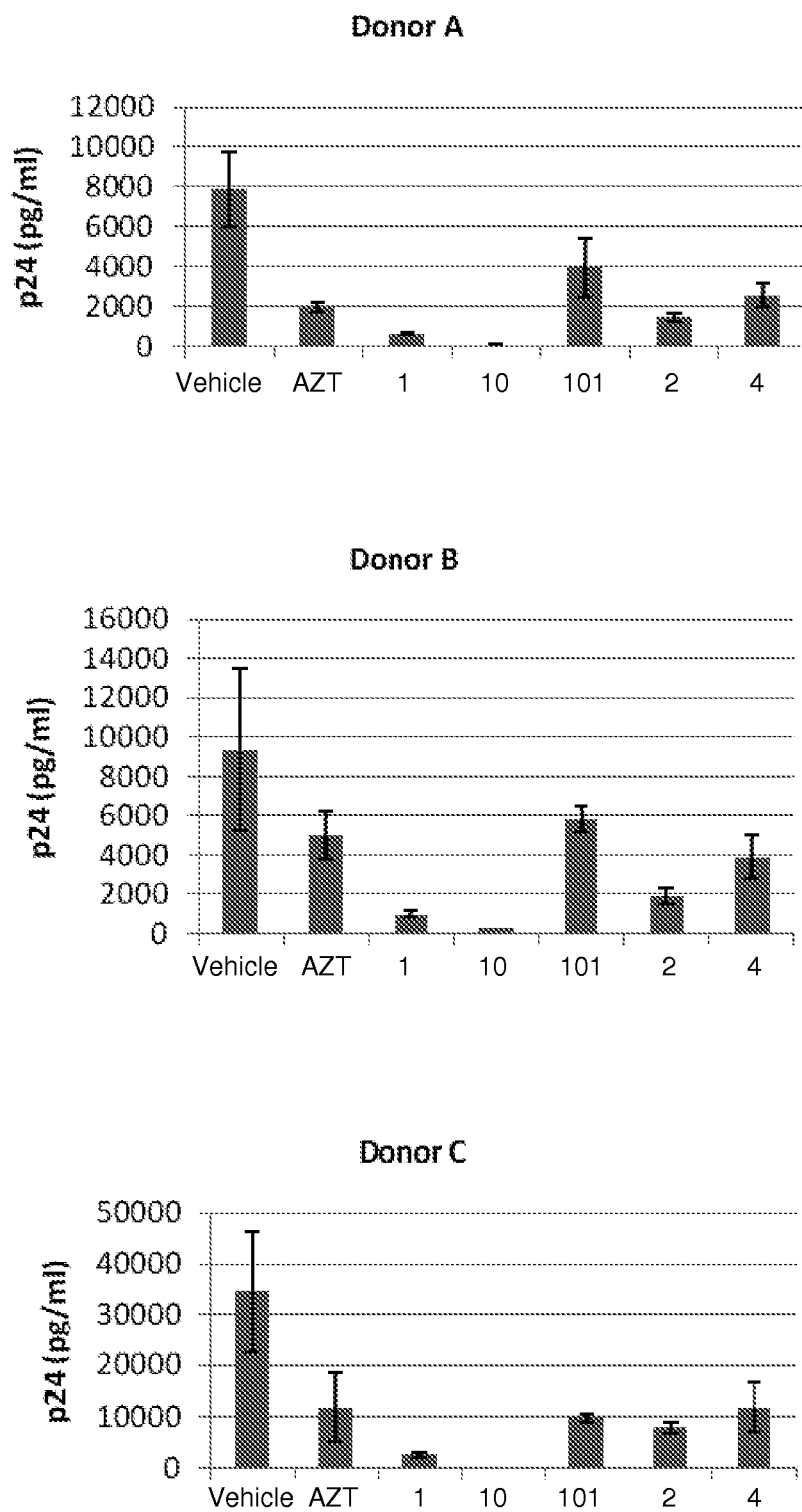
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**FIG. 1**

**FIG. 2**

**FIG. 3**

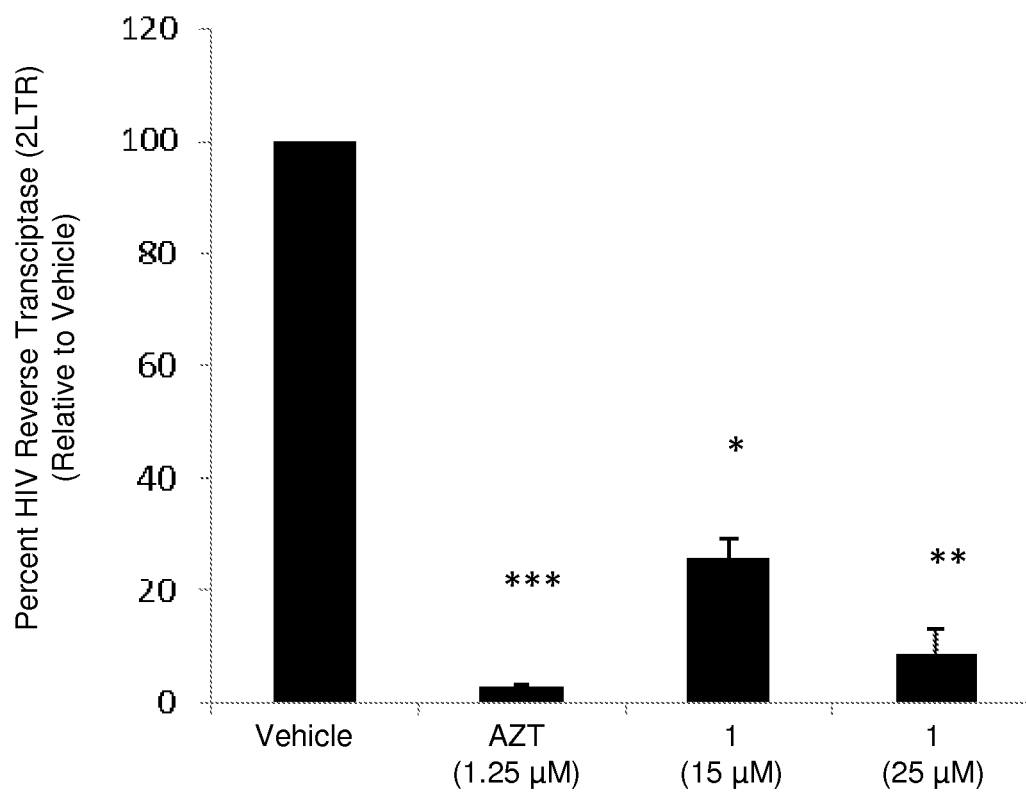


FIG. 4

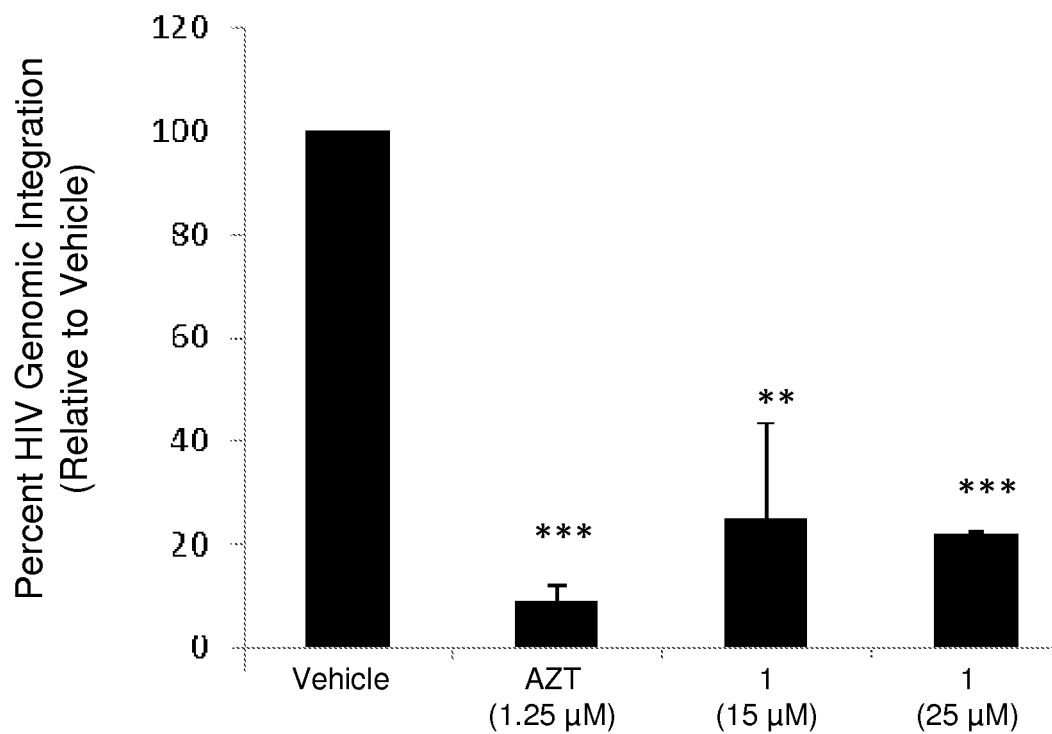


FIG. 5

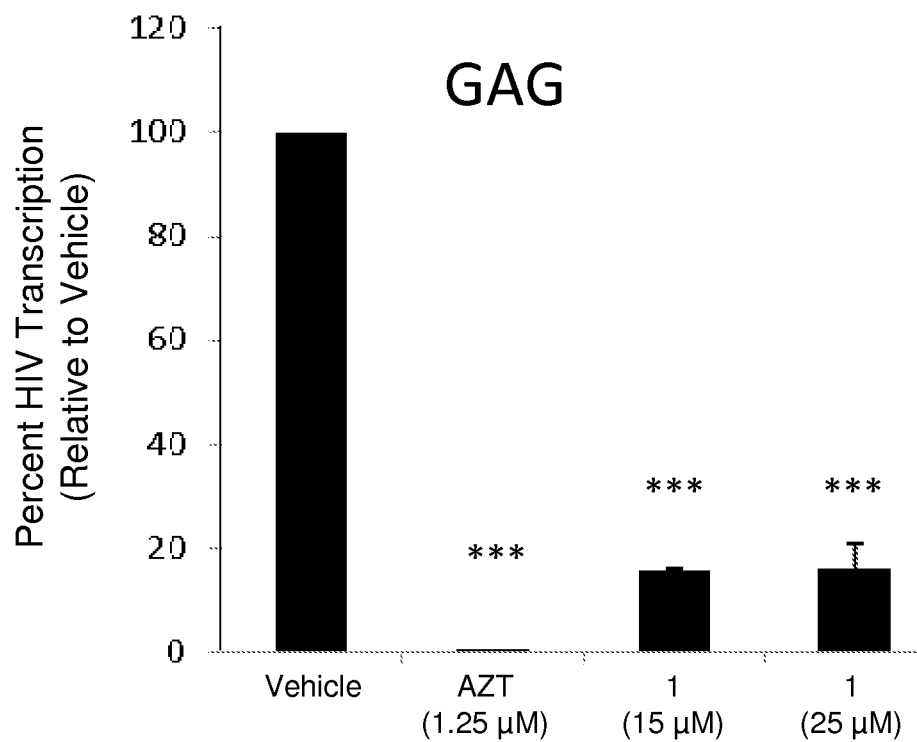
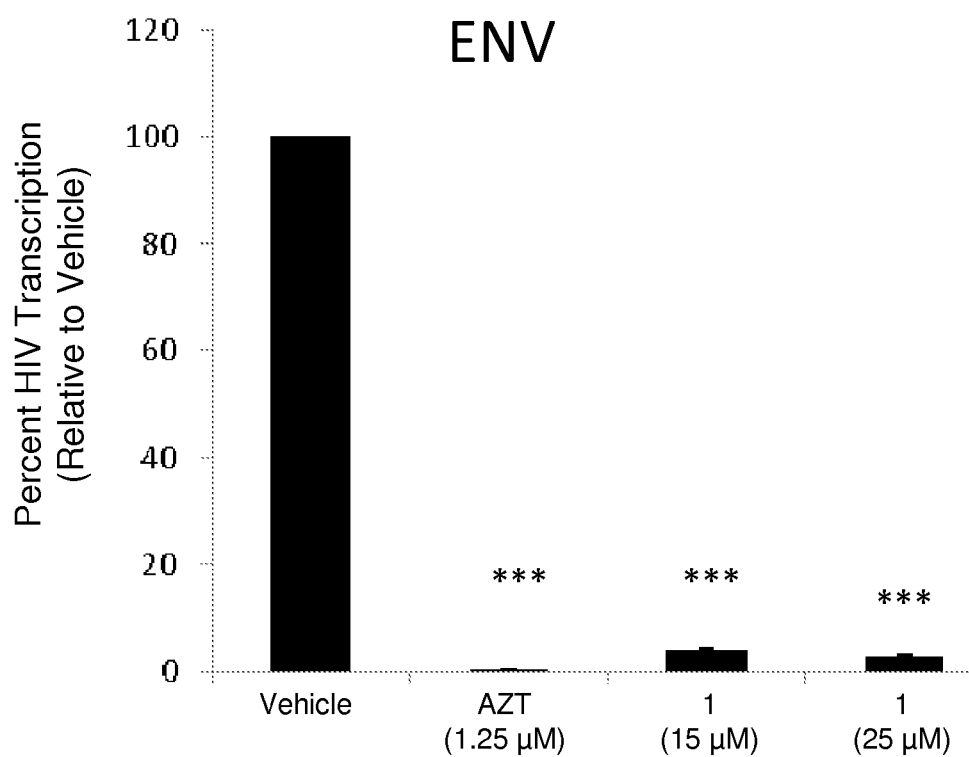
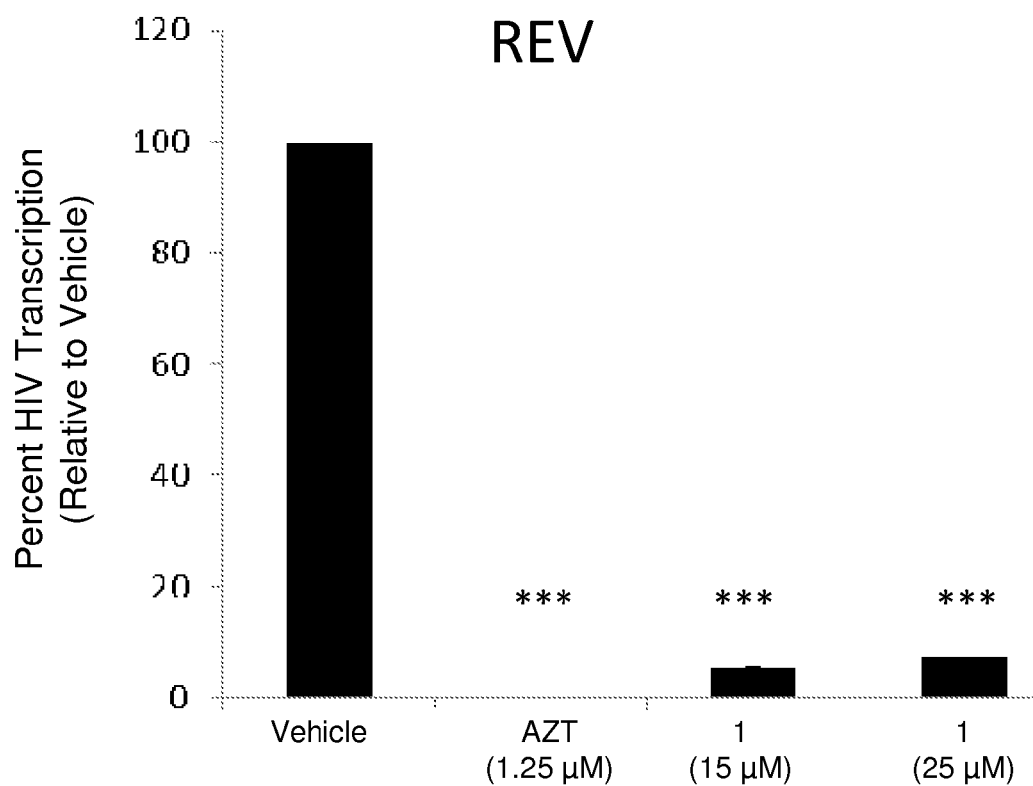
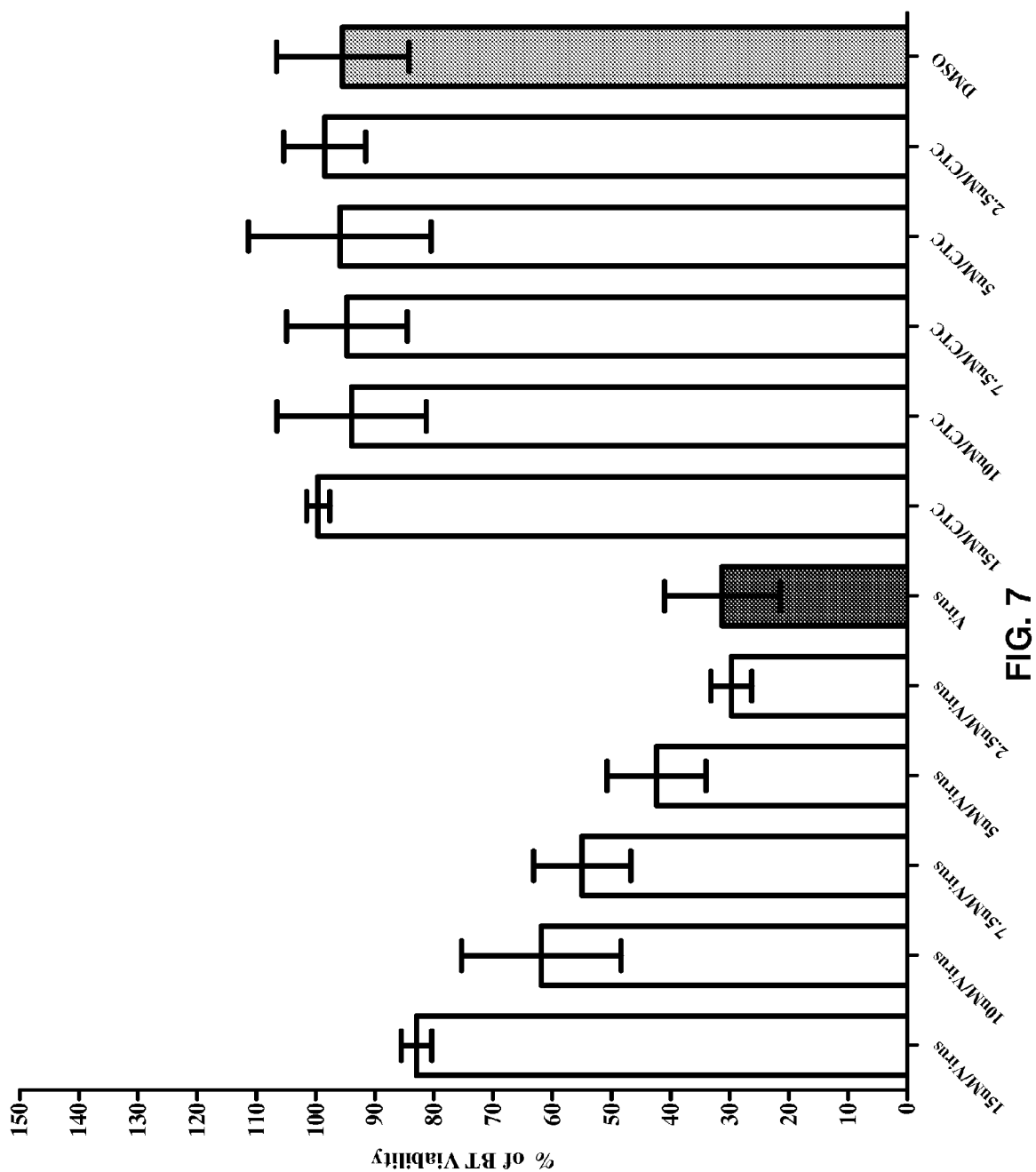
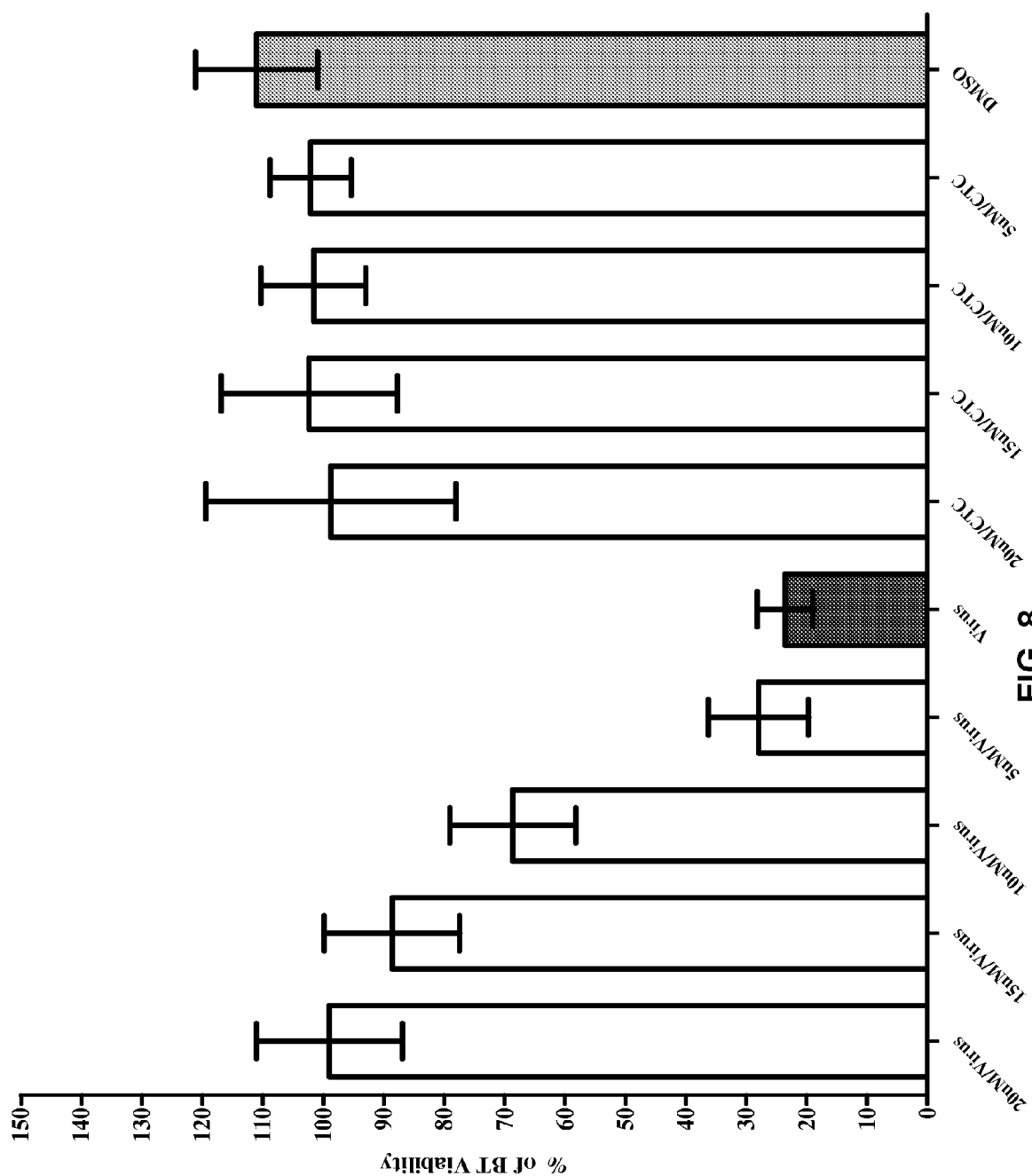
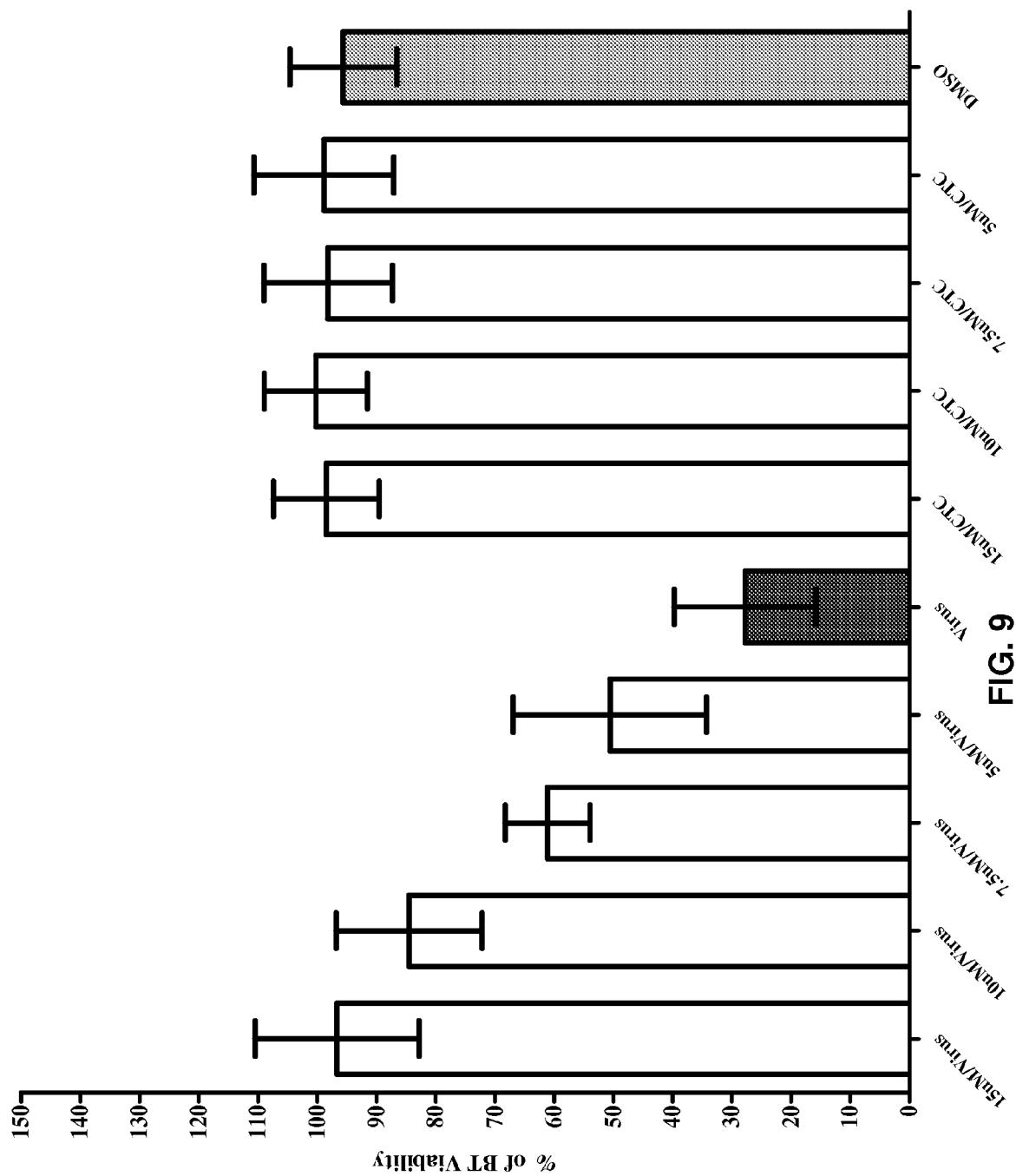


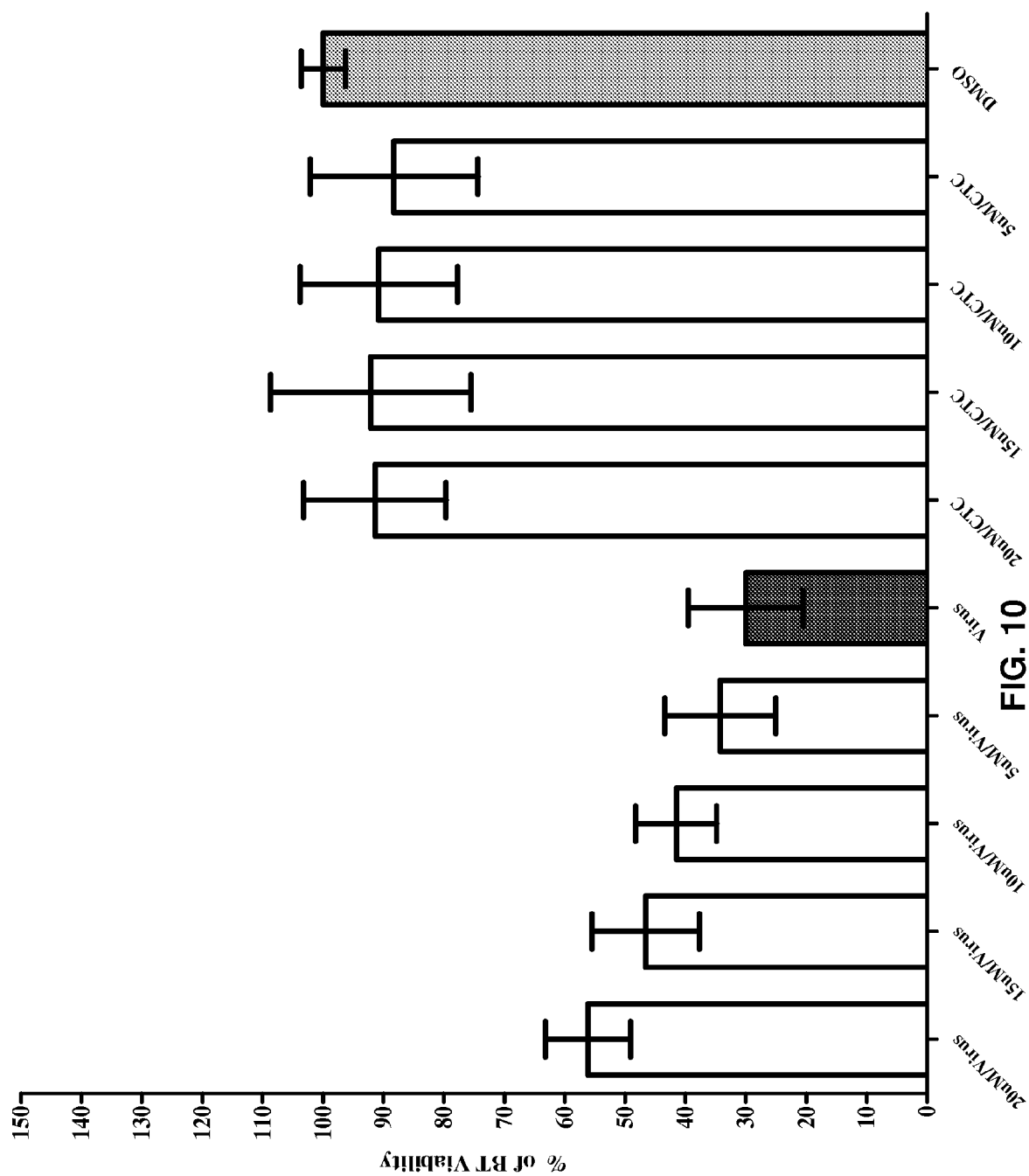
FIG. 6A

**FIG. 6B****FIG. 6C**









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COMPOSITIONS AND METHODS FOR TREATING VIRAL DISEASES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a U.S. nationalization under 35 U.S.C. §371 of International Application No. PCT/US2012/052108, filed Aug. 23, 2012, which claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Application No. 61/526,908, filed Aug. 24, 2011. The disclosures set forth in the referenced applications are incorporated herein by reference in their entirety, including all information as originally submitted to the United States Patent and Trademark Office

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Aug. 23, 2012, is named 119221_SEQ_ST25.txt and is 2,953 bytes in size.

TECHNICAL FIELD

The invention described herein pertains to substituted perhydro pyrrolopyridines and methods for their use in treating viral infections. In particular, the invention described herein pertains to substituted perhydro pyrrolopyridines and methods for their use in treating HIV infections, AIDS, and AIDS-related diseases, and BVDV infections.

BACKGROUND AND SUMMARY OF THE INVENTION

Acquired immune deficiency syndrome (AIDS) (1) is a disease caused at least in part by the retrovirus human immunodeficiency virus (HIV) (2). AIDS and AIDS-related diseases cause a gradual decline of the immune system and leaves the HIV-infected individuals susceptible to opportunistic infections and to tumor formation that eventually leads to death. Current therapies for these diseases include antiretroviral therapy, which generally involves a cocktail of HIV protease and reverse transcriptase inhibitor drugs. It has been reported that the therapy affords a significant improvement in the general health and quality of life of many HIV-infected individuals. That recovery is also associated with a marked reduction in HIV-associated morbidity and mortality (3). Even so, the HIV protease and reverse transcriptase inhibitor drug cocktail does not cure the patient of the HIV infection nor does it prevent the return of AIDS, once the treatment is stopped. It has been reported that patients who withdraw from the therapy do not benefit from the life-saving treatment. Moreover, for a considerable fraction of AIDS patients this treatment achieves far less than optimal results due to a number of factors, including therapy intolerance, unwanted side effects, other infections, and most notably the development of drug-resistant HIV strains (4). There is a continuing need for additional treatments for AIDS, AIDS-related diseases, and HIV infections.

A member of the Flaviviridae family of positive-sense, single-stranded RNA viruses is the Bovine Viral Diarrhea Virus (BVDV). Infection with this virus brings about a severe mucosal disease in cattle and other ruminants as well as pigs. BVDV cattle infections are marked by nose, mouth and gastrointestinal mucosa ulceration, which cause continuous salivation, nasal discharge, coughing and/or diarrhea. As a result

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there is a quick virus spread among animals. The virus also causes calves to be still born, become persistently infected, or suffer growth retardation and/or display severe neurological malformations. The economic impact of BVDV is considerable, although it is difficult to precisely estimate its level since certain infections remain undiagnosed or the losses are not recognized as being due to the virus. (see for example, Buckwold et al., *Antivirus Research* 2003, 60, 1; Finkielsztein et al., 2010, *Current Medicinal Chemistry* 17, 2933; foregoing publications, and each additional publication cited herein, are incorporated herein by reference). Thus effective therapeutics will be useful for reducing the economic impact of BVDV. Other members of this Flaviviridae family of diseases include West Nile Virus and Dengue Fever.

It has been discovered herein that perhydro pyrrolopyridines are active anti-HIV agents. It has also been discovered herein that perhydro pyrrolopyridines are active against Flaviviridae viruses and related diseases. It has also been discovered herein that perhydro pyrrolopyridines are active anti-BVDV agents. It has also been discovered herein that perhydro pyrrolopyridines are active anti-BVDV agents.

In one illustrative embodiment, described herein are substituted perhydro pyrrolopyridines that are useful for the treatment of HIV infections, AIDS, and AIDS-related diseases. In another embodiment, described herein are pharmaceutical compositions comprising the substituted perhydro pyrrolopyridines that are useful for the treatment of HIV infections, AIDS, and AIDS-related diseases. Illustratively, the compositions include one or more carriers, diluents, or excipients, or a combination thereof.

In another embodiment, described herein are methods for treating HIV infections, AIDS, and AIDS-related diseases, where the methods include administering the substituted perhydro pyrrolopyridines and/or the pharmaceutical compositions including the substituted perhydro pyrrolopyridines. In another embodiment, described herein is the use of one or more of the substituted perhydro pyrrolopyridines and/or the pharmaceutical compositions including the substituted perhydro pyrrolopyridines in the manufacture of a medicament for treating a patient or host animal having an HIV infection, AIDS, and AIDS-related diseases.

In another illustrative embodiment, described herein are substituted perhydro pyrrolopyridines that are useful for the treatment of BVDV infections. In another embodiment, described herein are pharmaceutical compositions comprising the substituted perhydro pyrrolopyridines that are useful for the treatment of BVDV infections. Illustratively, the compositions include one or more carriers, diluents, or excipients, or a combination thereof.

In another embodiment, described herein are methods for treating BVDV infections, where the methods include administering the substituted perhydro pyrrolopyridines and/or the pharmaceutical compositions including the substituted perhydro pyrrolopyridines. In another embodiment, described herein is the use of one or more of the substituted perhydro pyrrolopyridines and/or the pharmaceutical compositions including the substituted perhydro pyrrolopyridines in the manufacture of a medicament for treating a patient or host animal having a BVDV infection.

In another illustrative embodiment, described herein are substituted perhydro pyrrolopyridines that are useful for the treatment of West Nile virus infections. In another embodiment, described herein are pharmaceutical compositions comprising the substituted perhydro pyrrolopyridines that are useful for the treatment of West Nile virus infections. Illus-

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tratively, the compositions include one or more carriers, diluents, or excipients, or a combination thereof.

In another embodiment, described herein are methods for treating West Nile virus infections, where the methods include administering the substituted perhydro pyrrolopyridines and/or the pharmaceutical compositions including the substituted perhydro pyrrolopyridines. In another embodiment, described herein is the use of one or more of the substituted perhydro pyrrolopyridines and/or the pharmaceutical compositions including the substituted perhydro pyrrolopyridines in the manufacture of a medicament for treating a patient or host animal having a West Nile virus infection.

In another illustrative embodiment, described herein are substituted perhydro pyrrolopyridines that are useful for the treatment of Dengue fever. In another embodiment, described herein are pharmaceutical compositions comprising the substituted perhydro pyrrolopyridines that are useful for the treatment of Dengue fever. Illustratively, the compositions include one or more carriers, diluents, or excipients, or a combination thereof.

In another embodiment, described herein are methods for treating Dengue fever, where the methods include administering the substituted perhydro pyrrolopyridines and/or the pharmaceutical compositions including the substituted perhydro pyrrolopyridines. In another embodiment, described herein is the use of one or more of the substituted perhydro pyrrolopyridines and/or the pharmaceutical compositions including the substituted perhydro pyrrolopyridines in the manufacture of a medicament for treating a patient or host animal having a Dengue fever.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the effect of Example 1 on HIV replication in IL-2 and phytohemagglutinin (PHA)-stimulated cultured human peripheral blood mononuclear cells (PBMC) infected with HIV

FIG. 2 shows the lack of an apparent effect of the five tested substituted perhydro pyrrolopyridines on the viability of IL-2- and PHA-stimulated PBMC. The PBMC were incubated for 6 days with the substituted perhydro pyrrolopyridines.

FIG. 3 shows substituted perhydro pyrrolopyridine-induced reduction in p24 antigen levels in HIV-infected IL-2- and PHA-stimulated PBMC from three different consented donors.

FIG. 4 shows that Example 1 significantly inhibits reverse transcription at each concentration.

FIG. 5 shows that Example 1 significantly inhibits viral integration at each concentration.

FIGS. 6A-C show that Example 1 significantly inhibits viral transcription at each concentration.

FIG. 7 shows the efficacy of Example 1 on BVDV and the lack of apparent cytotoxicity in BT cells.

FIG. 8 shows the efficacy of Example 4 on BVDV and the lack of apparent cytotoxicity in BT cells.

FIG. 9 shows the efficacy of Example 10 on BVDV and the lack of apparent cytotoxicity in BT cells.

FIG. 10 shows the efficacy of Example 101 on BVDV and the lack of apparent cytotoxicity in BT cells.

DETAILED DESCRIPTION

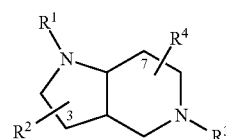
It has been discovered herein that substituted perhydro pyrrolopyridines, including octahydro-1H-pyrrolo[3,2-c]pyridines, are useful in treating viral diseases, such as HIV infections and/or BVDV infections. Without being bound by

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theory, it is believed herein that substituted perhydro pyrrolopyridines decrease viral load in infected cells.

It is to be understood that as used herein, the term "perhydro pyrrolopyridines", as well as the various embodiments represented by the formulae described herein, generally refers to the parent compounds as well as pharmaceutically acceptable salts thereof, including acid and/or base addition salts. In addition, it is to be understood that the term perhydro pyrrolopyridines includes various prodrugs of the compounds, as are described herein.

In one embodiment, described herein is a compound for treating a patient or host animal having HIV, said compound of formula I



(I)

or a pharmaceutically acceptable salt thereof, wherein:

R¹ is arylalkyl or arylacyl, each of which is optionally substituted;

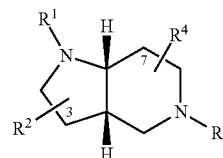
R² is hydrogen, alkyl, heteroalkyl, cycloalkyl, cycloheteroalkyl, aryl, or arylalkyl, each of which is optionally substituted;

R³ is arylalkyl or arylacyl, each of which is optionally substituted; and

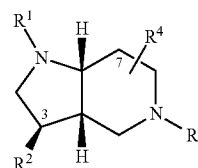
R⁴ is hydrogen, alkyl, heteroalkyl, cycloalkyl, cycloheteroalkyl, aryl, or arylalkyl, each of which is optionally substituted.

In another embodiment, described herein is a pharmaceutical composition comprising the compound of formula I above, or a pharmaceutically acceptable salt thereof.

In another embodiment, the stereochemistry of the ring fusion of the compound of formula I is syn. In another embodiment, the stereochemistry of the ring fusion of the compound of formula I is as follows:

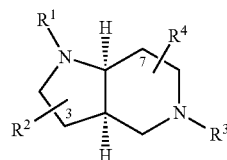


including pharmaceutically acceptable salts thereof. In another embodiment, the stereochemistry of the ring fusion and C-3 of the compound of formula I are as follows:

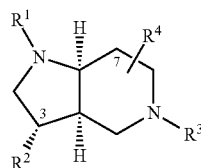


including pharmaceutically acceptable salts thereof. In another embodiment, the stereochemistry of the ring fusion of the compound of formula I is as follows:

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including pharmaceutically acceptable salts thereof. In another embodiment, the stereochemistry of the ring fusion and C-3 of the compound of formula I is as follows:



including pharmaceutically acceptable salts thereof.

In another embodiment, the substituents R^1 , R^2 , R^3 , and R^4 of the compounds of formula I described herein, including all stereochemical variations of the compounds described herein, are each independently selected from the following hereinbelow. In another embodiment, the substituent R^1 of the compound of formula I described herein is optionally substituted arylacyl. In another embodiment, the substituent R^1 of the compound of formula I described herein is an optionally substituted arylcarbonyl. In another embodiment, the substituent R^1 of the compound of formula I described herein is an optionally substituted benzoyl. In another embodiment, the substituent R^1 of the compound of formula I described herein is benzoyl. In another embodiment, the substituent R^1 of the compound of formula I described herein is optionally substituted picolinoyl. In another embodiment, the substituent R^1 of the compound of formula I described herein is picolinoyl. In another embodiment, the substituent R^2 of the compound of formula I described herein is optionally substituted aryl. In another embodiment, the substituent R^2 of the compound of formula I described herein is optionally substituted phenyl. In another embodiment, the substituent R^2 of the compound of formula I described herein is phenyl substituted with halo, alkoxy, or a combination thereof. In another embodiment, the substituent R^3 of the compound of formula I described herein is optionally substituted arylalkyl. In another embodiment, the substituent R^3 of the compound of formula I described herein is optionally substituted arylmethylene. In another embodiment, the substituent R^3 of the compound of formula I described herein is optionally substituted benzyl. In another embodiment, the substituent R^3 of the compound of formula I described herein is benzyl substituted with an electron withdrawing group. In another embodiment, the substituent R^3 of the compound of formula I described herein is halo substituted benzyl. In another embodiment, the substituent R^4 of the compound of formula I described herein is hydrogen or alkyl. In another embodiment, the substituent R^4 of the compound of formula I described herein is hydrogen. In another embodiment, the substituent R^2 of the compound of formula I described herein is at C-3.

In one embodiment, described herein are pharmaceutical compositions comprising one or more of the substituted perhydro pyrrolopyridines. The substituted perhydro pyrrolopyridines and the pharmaceutical compositions comprising

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them are useful in the treatment of HIV diseases. In another embodiment, the substituted perhydro pyrrolopyridines and the pharmaceutical compositions comprising them are useful in the treatment of BVDV diseases.

In another embodiment, described herein are methods of use of the substituted perhydro pyrrolopyridines and the pharmaceutical compositions comprising them for treating viral infections. Illustratively, these methods include administering to a patient or host animal in need of relief from the viral infection a therapeutically effective amount of one or more of the substituted perhydro pyrrolopyridines and/or the pharmaceutical compositions comprising them. In one variation, the methods described herein also include co-therapies with other therapeutic agents known to be useful in treating HIV. Accordingly, the compounds, compositions, formulations, uses, and methods described herein may be combined with any one or more of such compounds or agents known for treating HIV infections. Accordingly, in another embodiment, the co-therapy includes the co-administration of one or more of the compounds described herein and one or more of the known compounds or agents known to be useful in treating viral infections including HIV.

In another variation, the methods described herein also include co-therapies with other therapeutic agents known to be useful in treating BVDV, West Nile virus, and Dengue fever. Accordingly, the compounds, compositions, formulations, uses, and methods described herein may be combined with any one or more of such compounds or agents known for treating BVDV, West Nile virus, and Dengue fever infections. Accordingly, in another embodiment, the co-therapy includes the co-administration of one or more of the compounds described herein and one or more of the known compounds or agents known to be useful in treating viral infections including BVDV, West Nile virus, and Dengue fever.

As used herein, the term "alkyl" includes a chain of carbon atoms, which is optionally branched. As used herein, the term "alkenyl" and "alkynyl" includes a chain of carbon atoms, which is optionally branched, and includes at least one double bond or triple bond, respectively. It is to be understood that alkynyl may also include one or more double bonds. It is to be further understood that in certain embodiments, alkyl is advantageously of limited length, including C_1 - C_{24} , C_1 - C_{12} , C_1 - C_8 , C_1 - C_6 , and C_1 - C_4 . Illustratively, such particularly limited length alkyl groups, including C_1 - C_8 , C_1 - C_6 , and C_1 - C_4 may be referred to as lower alkyl. It is to be further understood that in certain embodiments alkenyl and/or alkynyl may each be advantageously of limited length, including C_2 - C_{24} , C_2 - C_{12} , C_2 - C_8 , C_2 - C_6 , and C_2 - C_4 . Illustratively, such particularly limited length alkenyl and/or alkynyl groups, including C_2 - C_8 , C_2 - C_6 , and C_2 - C_4 may be referred to as lower alkenyl and/or alkynyl. It is appreciated herein that shorter alkyl, alkenyl, and/or alkynyl groups may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior. Illustrative alkyl groups are, but not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, 3-pentyl, neopentyl, hexyl, heptyl, octyl, and the like.

As used herein, the term "alkylene" includes a divalent chain of carbon atoms, which is optionally branched. As used herein, the term "alkenylene" and "alkynylene" includes a divalent chain of carbon atoms, which is optionally branched, and includes at least one double bond or triple bond, respectively. It is to be understood that alkynylene may also include one or more double bonds. It is to be further understood that in certain embodiments, alkylene is advantageously of limited length, including C_1 - C_{24} , C_1 - C_{12} , C_1 - C_8 , C_1 - C_6 , and C_1 - C_4 . Illustratively, such particularly limited length alkylene

groups, including C₁-C₈, C₁-C₆, and C₁-C₄ may be referred to as lower alkylene. It is to be further understood that in certain embodiments alkenylene and/or alkynylene may each be advantageously of limited length, including C₂-C₂₄, C₂-C₁₂, C₂-C₈, C₂-C₆, and C₂-C₄. Illustratively, such particularly limited length alkenylene and/or alkynylene groups, including C₂-C₈, C₂-C₆, and C₂-C₄ may be referred to as lower alkenylene and/or alkynylene. It is appreciated herein that shorter alkylene, alkenylene, and/or alkynylene groups may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior. Illustrative alkyl groups are, but not limited to, methylene, ethylene, n-propylene, isopropylene, n-butylene, isobutylene, sec-butylene, pentylene, 1,2-pentylene, 1,3-pentylene, hexylene, heptylene, octylene, and the like.

As used herein, the term "cycloalkyl" includes a chain of carbon atoms, which is optionally branched, where at least a portion of the chain is cyclic. It is to be understood that cycloalkylalkyl is a subset of cycloalkyl. It is to be understood that cycloalkyl may be polycyclic. Illustrative cycloalkyl include, but are not limited to, cyclopropyl, cyclopentyl, cyclohexyl, 2-methylcyclopropyl, cyclopentyleth-2-yl, adamantyl, and the like. As used herein, the term "cycloalkenyl" includes a chain of carbon atoms, which is optionally branched, and includes at least one double bond, where at least a portion of the chain is cyclic. It is to be understood that the one or more double bonds may be in the cyclic portion of cycloalkenyl and/or the non-cyclic portion of cycloalkenyl. It is to be understood that cycloalkenylalkyl and cycloalkylalkenyl are each subsets of cycloalkenyl. It is to be understood that cycloalkyl may be polycyclic. Illustrative cycloalkenyl include, but are not limited to, cyclopentenyl, cyclohexylethen-2-yl, cycloheptenylpropenyl, and the like. It is to be further understood that chain forming cycloalkyl and/or cycloalkenyl is advantageously of limited length, including C₃-C₂₄, C₃-C₁₂, C₃-C₈, C₃-C₆, and C₃-C₄. It is appreciated herein that shorter alkyl and/or alkenyl chains forming cycloalkyl and/or cycloalkenyl, respectively, may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior.

As used herein, the term "heteroalkyl" includes a chain of atoms that includes both carbon and at least one heteroatom, and is optionally branched. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. As used herein, the term "cycloheteroalkyl" including heterocyclyl and heterocycle, includes a chain of atoms that includes both carbon and at least one heteroatom, such as heteroalkyl, and is optionally branched, where at least a portion of the chain is cyclic. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. Illustrative cycloheteroalkyl include, but are not limited to, tetrahydrofuryl, pyrrolidinyl, tetrahydropyranlyl, piperidinyl, morpholinyl, piperazinyl, homopiperazinyl, quinuclidinyl, and the like.

As used herein, the term "aryl" includes monocyclic and polycyclic aromatic carbocyclic groups, each of which may be optionally substituted. Illustrative aromatic carbocyclic groups described herein include, but are not limited to, phenyl, naphthyl, and the like. As used herein, the term "heteroaryl" includes aromatic heterocyclic groups, each of which may be optionally substituted. Illustrative aromatic heterocyclic groups include, but are not limited to, pyridinyl, pyrimidinyl, pyrazinyl, triazinyl, tetrazinyl, quinolinyl, quinoxalinyl, thienyl, pyrazolyl, imidazolyl, oxazolyl, thiazolyl, isoxazolyl, isothiazolyl, oxadiazolyl,

thiadiazolyl, triazolyl, benzimidazolyl, benzoxazolyl, benzthiazolyl, benzisoxazolyl, benzisothiazolyl, and the like.

As used herein, the term "amino" includes the group NH₂, alkylamino, and dialkylamino, where the two alkyl groups in dialkylamino may be the same or different, i.e. alkylalkylamino. Illustratively, amino includes methylamino, ethylamino, dimethylamino, methylethylamino, and the like. In addition, it is to be understood that when amino modifies or is modified by another term, such as aminoalkyl, or acylamino, the above variations of the term amino are included therein. Illustratively, aminoalkyl includes H₂N-alkyl, methylaminoalkyl, ethylaminoalkyl, dimethylaminoalkyl, methylethylaminoalkyl, and the like. Illustratively, acylamino includes acylmethylamino, acylethylamino, and the like.

As used herein, the term "amino and derivatives thereof" includes amino as described herein, and alkylamino, alkenylamino, alkynylamino, heteroalkylamino, heteroalkenylamino, heteroalkynylamino, cycloalkylamino, cycloalkenylamino, cycloheteroalkylamino, cycloheteroalkenylamino, arylamino, arylalkylamino, arylalkenylamino, arylalkynylamino, heteroarylaminomino, heteroarylalkylamino, heteroarylalkenylamino, heteroarylalkynylamino, acylamino, and the like, each of which is optionally substituted. The term "amino derivative" also includes urea, carbamate, and the like.

As used herein, the term "hydroxy and derivatives thereof" includes OH, and alkyloxy, alkenyloxy, alkynyloxy, heteroalkyloxy, heteroalkenyloxy, heteroalkynyloxy, cycloalkyloxy, cycloalkenyloxy, cycloheteroalkyloxy, cycloheteroalkenyloxy, aryloxy, arylalkyloxy, arylalkenyloxy, arylalkynyloxy, heteroaryloxy, heteroarylalkyloxy, heteroarylalkenyloxy, heteroarylalkynyloxy, acyloxy, and the like, each of which is optionally substituted. The term "hydroxy derivative" also includes carbamate, and the like.

As used herein, the term "thio and derivatives thereof" includes SH, and alkylthio, alkenylthio, alkynylthio, heteroalkylthio, heteroalkenylthio, heteroalkynylthio, cycloalkylthio, cycloalkenylthio, cycloheteroalkylthio, cycloheteroalkenylthio, arylthio, arylalkylthio, arylalkenylthio, arylalkynylthio, heteroarylthio, heteroarylalkylthio, heteroarylalkenylthio, heteroarylalkynylthio, acylthio, and the like, each of which is optionally substituted. The term "thio derivative" also includes thiocarbamate, and the like.

As used herein, the term "acyl" includes formyl, and alkylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, heteroalkylcarbonyl, heteroalkenylcarbonyl, heteroalkynylcarbonyl, cycloalkylcarbonyl, cycloalkenylcarbonyl, cycloheteroalkylcarbonyl, cycloheteroalkenylcarbonyl, arylcarbonyl, arylalkylcarbonyl, arylalkenylcarbonyl, arylalkynylcarbonyl, heteroarylcarbonyl, heteroarylalkylcarbonyl, heteroarylalkenylcarbonyl, heteroarylalkynylcarbonyl, acylcarbonyl, and the like, each of which is optionally substituted.

As used herein, the term "carbonyl and derivatives thereof" includes the group C(O), C(S), C(NH) and substituted amino derivatives thereof.

As used herein, the term "carboxylic acid and derivatives thereof" includes the group CO₂H and salts thereof, and esters and amides thereof, and CN.

As used herein, the term "sulfonyl" includes alkylsulfonyl, alkenylsulfonyl, alkynylsulfonyl, heteroalkylsulfonyl, heteroalkenylsulfonyl, heteroalkynylsulfonyl, cycloalkylsulfonyl, cycloalkenylsulfonyl, cycloheteroalkylsulfonyl, cycloheteroalkenylsulfonyl, arylsulfonyl, arylalkylsulfonyl, arylalkenylsulfonyl, arylalkynylsulfonyl, heteroarylsulfonyl, heteroarylalkylsulfonyl, heteroarylalkenylsulfonyl, heteroarylalkynylsulfonyl, acylsulfonyl, and the like, each of which is optionally substituted.

Prodrugs may be prepared from the compounds described herein by attaching groups that ultimately cleave in vivo to

It is understood that the prodrugs themselves may not possess significant biological activity, but instead undergo one or more spontaneous chemical reaction(s), enzyme-catalyzed chemical reaction(s), and/or metabolic chemical reaction(s), or a combination thereof after administration in vivo to produce the compound described herein that is biologically active or is a precursor of the biologically active compound. However, it is appreciated that in some cases, the prodrug is biologically active. It is also appreciated that prodrugs may often serve to improve drug efficacy or safety through improved oral bioavailability, pharmacodynamic half-life, and the like. Prodrugs also refer to derivatives of the compounds described herein that include groups that simply mask undesirable drug properties or improve drug delivery. For example, one or more compounds described herein may exhibit an undesirable property that is advantageously blocked or minimized may become pharmacological, pharmaceutical, or pharmacokinetic barriers in clinical drug application, such as low oral drug absorption, lack of site

specificity, chemical instability, toxicity, and poor patient acceptance (bad taste, odor, pain at injection site, and the like), and others. It is appreciated herein that a prodrug, or other strategy using reversible derivatives, can be useful in the optimization of the clinical application of a drug.

As used herein, the term "composition" generally refers to any product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combinations of the specified ingredients in the specified amounts. It is to be understood that the compositions described herein may be prepared from isolated compounds described herein or from salts, solutions, hydrates, solvates, and other forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various amorphous, non-amorphous, partially crystalline, crystalline, and/or other morphological forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various hydrates and/or solvates of the compounds described herein. Accordingly, such pharmaceutical compositions that recite compounds described herein are to be understood to include each of, or any combination of, the various morphological forms and/or solvate or hydrate forms of the compounds described herein. Illustratively, compositions may include one or more carriers, diluents, and/or excipients. The compounds described herein, or compositions containing them, may be formulated in a therapeutically effective amount in any conventional dosage forms appropriate for the methods described herein. The compounds described herein, or compositions containing them, including such formulations, may be administered by a wide variety of conventional routes for the methods described herein, and in a wide variety of dosage formats, utilizing known procedures (see generally, Remington: The Science and Practice of Pharmacy, (21st ed., 2005)).

The term "therapeutically effective amount" as used herein, refers to that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated. In one aspect, the therapeutically effective amount is that which may treat or alleviate the disease or symptoms of the disease at a reasonable benefit/risk ratio applicable to any medical treatment. However, it is to be understood that the total daily usage of the compounds and compositions described herein may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors, including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, gender and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidentally with the specific compound employed; and like factors well known to the researcher, veterinarian, medical doctor or other clinician of ordinary skill.

It is also appreciated that the therapeutically effective amount, whether referring to monotherapy or combination therapy, is advantageously selected with reference to any toxicity, or other undesirable side effect, that might occur during administration of one or more of the compounds described herein. Further, it is appreciated that the co-therapies described herein may allow for the administration of lower doses of compounds that show such toxicity, or other

undesirable side effect, where those lower doses are below thresholds of toxicity or lower in the therapeutic window than would otherwise be administered in the absence of a cotherapy.

In addition to the illustrative dosages and dosing protocols described herein, it is to be understood that an effective amount of any one or a mixture of the compounds described herein can be readily determined by the attending diagnostician or physician by the use of known techniques and/or by observing results obtained under analogous circumstances. In determining the effective amount or dose, a number of factors are considered by the attending diagnostician or physician, including, but not limited to the species of mammal, including human, its size, age, and general health, the specific disease or disorder involved, the degree of or involvement or the severity of the disease or disorder, the response of the individual patient, the particular compound administered, the mode of administration, the bioavailability characteristics of the preparation administered, the dose regimen selected, the use of concomitant medication, and other relevant circumstances.

The dosage of each compound of the claimed combinations depends on several factors, including: the administration method, the condition to be treated, the severity of the condition, whether the condition is to be treated or prevented, and the age, weight, and health of the person to be treated. Additionally, pharmacogenomic (the effect of genotype on the pharmacokinetic, pharmacodynamic or efficacy profile of a therapeutic) information about a particular patient may affect the dosage used.

It is to be understood that in the methods described herein, the individual components of a co-administration, or combination can be administered by any suitable means, contemporaneously, simultaneously, sequentially, separately or in a single pharmaceutical formulation. Where the co-administered compounds or compositions are administered in separate dosage forms, the number of dosages administered per day for each compound may be the same or different. The compounds or compositions may be administered via the same or different routes of administration. The compounds or compositions may be administered according to simultaneous or alternating regimens, at the same or different times during the course of the therapy, concurrently in divided or single forms.

The term "administering" as used herein includes all means of introducing the compounds and compositions described herein to the patient, including, but are not limited to, oral (po), intravenous (iv), intramuscular (im), subcutaneous (sc), transdermal, inhalation, buccal, ocular, sublingual, vaginal, rectal, and the like. The compounds and compositions described herein may be administered in unit dosage forms and/or formulations containing conventional nontoxic pharmaceutically-acceptable carriers, adjuvants, and vehicles.

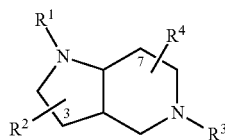
Illustrative formats for oral administration include tablets, capsules, elixirs, syrups, and the like.

Illustrative routes for parenteral administration include intravenous, intraarterial, intraperitoneal, epidural, intraurethral, intrasternal, intramuscular and subcutaneous, as well as any other art recognized route of parenteral administration.

Several illustrative embodiments of the invention are described by the following enumerated clauses:

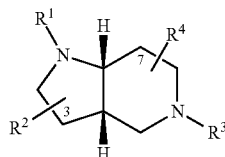
1. A composition for treating a host animal with HIV or a related disease, BVDV, West Nile virus, or Dengue fever, or other Flaviviridae viral infection or related disease, or a combination of the foregoing, said composition comprising a compound of the formula

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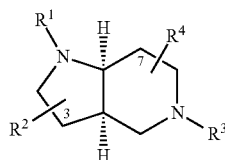
or a pharmaceutically acceptable salt thereof, wherein:

- R¹ is arylalkyl or arylacyl, each of which is optionally substituted;
 - R² is hydrogen, alkyl, heteroalkyl, cycloalkyl, cycloheteroalkyl, aryl, or arylalkyl, each of which is optionally substituted;
 - R³ is arylalkyl or arylacyl, each of which is optionally substituted; and
 - R⁴ is hydrogen, alkyl, heteroalkyl, cycloalkyl, cycloheteroalkyl, aryl, or arylalkyl, each of which is optionally substituted.
2. The composition of clause 1 wherein the stereochemistry of the ring fusion in the compound is syn.
 3. The composition of clause 1 wherein the compound is of the formula



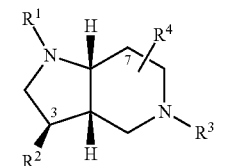
or a pharmaceutically acceptable salt thereof.

- 3A. The composition of clause 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

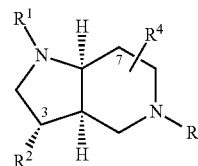
4. The composition of clause 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

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- 4A. The composition of clause 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

5. The composition of any one of clauses 1 to 5 wherein R¹ is optionally substituted arylalkyl.
6. The composition of any one of clauses 1 to 5 wherein R¹ is optionally substituted arylcarbonyl.
7. The composition of any one of clauses 1 to 5 wherein R¹ is optionally substituted benzoyl.
8. The composition of any one of clauses 1 to 5 wherein R¹ is benzoyl.
9. The composition of any one of clauses 1 to 5 wherein R¹ is optionally substituted picolinoyl.
10. The composition of any one of clauses 1 to 5 wherein R¹ is picolinoyl.
11. The composition of any one of clauses 1 to 5 wherein R¹ is 3-picolinoyl.
12. The composition of any one of clauses 1 to 11 wherein R² is optionally substituted aryl.
13. The composition of any one of clauses 1 to 11 wherein R² is optionally substituted phenyl.
14. The composition of any one of clauses 1 to 11 wherein R² is phenyl substituted with halo, alkoxy, or a combination thereof.
15. The composition of any one of clauses 1 to 11 wherein R² is halophenyl.
16. The composition of any one of clauses 1 to 11 wherein R² is alkoxyphenyl.
17. The composition of any one of clauses 1 to 11 wherein R² is phenyl substituted with an electron donating group.
18. The composition of any one of clauses 1 to 11 wherein R² is methoxyphenyl.
19. The composition of any one of clauses 1 to 18 wherein R³ is optionally substituted arylalkyl.
20. The composition of any one of clauses 1 to 18 wherein R³ is optionally substituted arylmethylene.
21. The composition of any one of clauses 1 to 18 wherein R³ is optionally substituted benzyl.
22. The composition of any one of clauses 1 to 18 wherein R³ is benzyl substituted with an electron withdrawing group.
23. The composition of any one of clauses 1 to 18 wherein R³ is halo substituted benzyl.
24. The composition of any one of clauses 1 to 18 wherein R³ is benzyl substituted fluoro, chloro, or a combination thereof.
25. The composition of any one of clauses 1 to 18 wherein R³ is fluorobenzyl.
26. The composition of any one of clauses 1 to 18 wherein R³ is chlorobenzyl.
27. The composition of any one of clauses 1 to 26 wherein R⁴ is hydrogen or alkyl.
28. The composition of any one of clauses 1 to 26 wherein R⁴ is hydrogen.
29. The composition of any one of clauses 1 to 28 wherein R² is at C-3.

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30. The composition of any one of clauses 1 to 29 for treating HIV.
31. The composition of any one of clauses 1 to 29 for treating West Nile virus.
32. The composition of any one of clauses 1 to 29 for treating Dengue fever.
33. The composition of any one of clauses 30 to 32 wherein the host animal is a human.
34. The composition of any one of clauses 1 to 29 for treating BVDV.
35. The composition of clause 34 wherein the host animal is a bovine.
36. Use of the compound of any one of clauses 1 to 29 in the manufacture of a medicament for treating a host animal with HIV.
37. A method for treating a host animal with HIV, the method comprising the step of administering to the host animal a therapeutically effective amount of one or more compounds from any one of clauses 1 to 29 alone or as a composition with one or more carriers, diluents, or excipients, or a combination thereof.
38. The method of clause 37 wherein the host animal is a human.
39. Use of the compound of any one of clauses 1 to 29 in the manufacture of a medicament for treating a host animal with BVDV.
40. A method for treating a host animal with BVDV, the method comprising the step of administering to the host animal a therapeutically effective amount of one or more compounds from any one of clauses 1 to 29 alone or as a composition with one or more carriers, diluents, or excipients, or a combination thereof.
41. The method of clause 40 wherein the host animal is a bovine.

In another embodiment, described herein is the use of one or more of the compounds described herein in the manufacture of a medicament for treating a patient or host animal having HIV, AIDS, or an AIDS-related disease, or a combination thereof.

In another embodiment, described herein is a method for treating a patient or host animal having HIV, AIDS, or an AIDS-related disease, or a combination thereof, the method comprising the step of administering to the patient or host animal a therapeutically effective amount of one or more of the compounds described herein alone or as a composition with one or more carriers, diluents, or excipients, or a combination thereof.

In another embodiment, described herein is the use of one or more of the compounds described herein in the manufacture of a medicament for treating a patient or host animal having a Flaviviridae infection or related disease.

In another embodiment, described herein is the use of one or more of the compounds described herein in the manufacture of a medicament for treating a patient or host animal having West Nile virus.

In another embodiment, described herein is the use of one or more of the compounds described herein in the manufacture of a medicament for treating a patient or host animal having Dengue fever.

In another embodiment, described herein is the use of one or more of the compounds described herein in the manufacture of a medicament for treating a patient or host animal having BVDV.

In another embodiment, described herein is a method for treating a patient or host animal having BVDV, the method comprising the step of administering to the patient or host animal a therapeutically effective amount of one or more of

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the compounds described herein alone or as a composition with one or more carriers, diluents, or excipients, or a combination thereof.

It is to be understood that each of the foregoing selections for R^1 , R^2 , R^3 , and R^4 are described herein for each of the stereochemical embodiments of formula I. It is to be further understood that each of the foregoing selections for R^1 , R^2 , R^3 , and R^4 may be combined with each other in every possible combination, each of which forms a description of a further illustrative embodiment of the invention. For example, in one such combination, described herein are compounds of formula I, including any of the stereochemical embodiments of formula I, where R^1 is an optionally substituted arylacyl and R^2 is optionally substituted aryl. In another such combination, described herein are compounds of formula I, including any of the stereochemical embodiments of formula I, where R^1 is an optionally substituted benzoyl; R^2 is optionally substituted aryl; and R^3 is optionally substituted arylalkyl. In another such combination, described herein are compounds of formula I, including any of the stereochemical embodiments of formula I, where R^1 is benzoyl; R^2 is optionally substituted aryl; R^3 is benzyl substituted with an electron withdrawing group; and R^4 is hydrogen.

The effective use of the compounds, compositions, and methods described herein for treating or ameliorating one or more effects of HIV, and/or BVDV using one or more compounds described herein may be based upon animal models, such as murine, canine, porcine, and non-human primate animal models of disease. For example, it is understood that HIV in humans may be characterized by a loss of function, and/or the development of symptoms, each of which may be elicited in animals, such as mice, and other surrogate test animals. Further, it is understood that BVDV in bovine may be characterized by a loss of function, and/or the development of symptoms, each of which may be elicited in alternative animals, such as mice, and other surrogate test animals. Such animal models may be used to evaluate the methods of treatment and the pharmaceutical compositions described herein to determine the therapeutically effective amounts described herein.

EXAMPLES

The following examples further illustrate specific embodiments of the invention; however, the following examples should not be interpreted in any way to limit the invention.

Example

Test Compounds. The substituted perhydropyrrolopyridines described herein are prepared using conventional processes, or are obtained from commercial suppliers (>90% purity) and used as is.

Example

Test compounds are evaluated using conventional assays for HIV infection, conventional assays for HIV protease activity, and conventional animal models of HIV disease.

Example

Blood Draw. 10 mL BD Vacutainer, heparin coated (#367874) tubes are used to collect blood from healthy donors after consent. Average of 10×10^6 PBMCs per 10 mL tube of blood is used to determine total amount of blood drawn from each donor.

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Example

Test compounds are evaluated using conventional assays for the viability of IL-2 and phytohaemagglutinin (PHA)-stimulated cultured human peripheral blood mononuclear cells (PBMC) in the presence of test compounds. Test compounds are evaluated using conventional assays on HIV replication in such cells infected with HIV. Control Cell viability is determined by a colormetric assay, such as MTS (Promega catalogue #G3582) and HIV replication by measuring the level of HIV capsid p24 antigen (5) using an ELISA kit supplied by the NIH. The substituted perhydropyrrolopyridines are dissolved in dimethyl sulfoxide (DMSO) and used at a final concentration of 25 μ M. The final DMSO concentration in the growth medium is 0.5%. Azidothymidine (AZT), at a final concentration of 1 μ M, serves as a positive control while DMSO in the absence of the substituted perhydropyrrolopyridines or AZT serves as a negative control.

Example

Isolation and stimulation of peripheral blood mononuclear cells (PBMCs). For the cell viability assay and P24 level assessment after substituted perhydropyrrolopyridine treatment, blood is collected from healthy consented donors using a 10 mL heparin coated tube (BD Vacutainer #367874). The collected blood containing about 10^7 PBMC is mixed with an equal amount of sterile phosphate buffered saline (PBS) in 50 mL tube. The blood/PBS mixture is then slowly overlaid onto 10 mL Ficoll solution (Lymphocyte separation medium) and centrifuged at 2000 rpm for 5 minutes to separate the PBMC layer from whole blood. To maintain separated layers, the centrifuge is allowed a slow stop (no brake). The PBMC are removed from the 50 mL centrifuge tube by aspiration using a sterile Pasteur pipette and placed into a 15 mL centrifuge tube to which PBS is added to fill the tube. To remove platelets, the tube is centrifuged at 2000 rpm for 5 min (with the break on) in order to pellet the freshly isolated PBMC. The supernatant with the platelets is decanted and PBS added to the tube to a 15 mL volume and centrifuged at 1400 rpm for 5 min. after which time the supernatant is decanted and the pellet suspended in 5 mL growth medium (RPMI-1640 medium plus 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin) with 20 units/ml IL-2 and 4 μ g/ml PHA and incubated for a day in T25 culture flasks at 37° C. and 5% CO₂ in a humidified incubator.

Example

Infection of PBMCs with HIV-1 Bal: HIV-1 Bal (NIH AIDS Research and Reference Reagent Program, Frederick, Md.) at a concentration of 2 ng virus/ 10^6 cells was added to stimulated PBMCs and incubated for 5 h at 37° C., in a 5% CO₂, humidified incubator. Cells were then washed with media three times. For p24 assays, 10^6 cells per condition were resuspended in 1 mL of RPMI complete medium with 20 units/mL IL-2. Cells were left untreated or treated with 1.25 μ M AZT (Sigma, St Louis, Mo.) and 1.25 μ M vehicle control (DMSO). For p24 assays, infected and treated cells were then plated in a 96 well U-bottom plate at 200,000 cells in 200 μ L volume of the media in quadruplicates for 6 days at 37° C., in a 5% CO₂, humidified incubator. For RNA isolation and subsequent real time PCR studies, 3×10^6 infected and washed cells were resuspended in RPMI complete medium with 20 units/mL of IL-2 and subjected to appropriate treatments as mentioned above and cultured at 1 mL volume in 12

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well culture plates for 1 to 3 days (depending on testing time points of HIV replication cycle) at 37° C., 5% CO₂ humidified incubator.

Example

HIV replication inhibition by the five substituted perhydropyrrolopyridines is determined by assaying their effect on the levels of the HIV capsid protein p24 (6) in HIV-infected PBMC. The amount of P24 is assayed by using a sandwich ELISA assay kit obtained from SAIC-Frederick AIDS (Frederick, Md.) reagent program and performed according to the manufacturer's protocol. The kit includes a coated plate, standards, primary and secondary antibodies. For this P24 determination, a day after stimulation of the PBMC with IL-2 and PHA, samples of the stimulated PBMC containing about 10^6 cells are centrifuged for 5 min at 2000 rpm. The supernatant is decanted and 2 ng of HIV-Bal virus stock provided by the NIH AIDS Reagent and Reference Program is added to each pellet and the cells suspended with growth medium to a total of 1 mL and incubated at 37° C. and 5% CO₂ in a humidified incubator. After 4 h, the cells are washed 3 times with 5 mL growth medium at 2000 rpm for 5 min. The final pellet is re-suspended in fresh growth medium to which 20 units/mL IL-2 is added. Aliquots of 1 mL PBMC suspension containing around 10^6 cells per mL are placed into 1.5 mL microcentrifuge tubes and treated with the test chemicals. For each treatment, 4 aliquots of 200 μ L (about 2×10^5 cells) are each dispensed into separate wells of a U-bottom 96 well plate and incubated at 37° C. and 5% CO₂ in a humidified incubator.

After 7 day incubation, the supernatant from each well is transferred to a flat bottom 96 well plate and lysed with a 1/10 volume of 10% Triton-x 100 in DI-H₂O at 37° C. for 1 h. The test plates are washed three times with 200 μ L of wash buffer. 100 μ L of standards at 40 ng/mL, 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.3125 ng/mL, 0.1563 ng/mL and 0 ng/mL of p24 lysate control as well as 100 μ L of test samples diluted 1:10 in 1% BSA, 0.2% Tween 20 in RPMI are added into the antigen-coated 96 well plate. The plates are incubated at 37° C. for 2 h and washed three times with 200 μ L wash buffer. 100 μ L of primary antibody solution diluted 1:150 in 10% fetal bovine serum, 2% normal mouse serum in RPMI medium is added to each well and the plates are incubated at 37° C. for 1 h. After three washes with 200 μ L wash buffer, 100 μ L of secondary antibody diluted 1:50 in 2% normal mouse serum, 5% NGS, 0.01% Tween 20 in RPMI medium is added. The plates are incubated at 37° C. for 1 h and washed three times with 200 μ L wash buffer. 100 μ L of TMB, prepared by mixing equal volumes of each component solution provided with the KPL kit (KPL, Gaithersburg, Md.), are added to each well and the plate is incubated at room temperature, in the dark for 30 min. The reaction is stopped by adding 100 μ L 1N NaCl and the plates are read using a plate reader at 450 nm with a 650 nm background. A four parameter analysis is used to calculate the standard curve and concentration based on absorbance readings.

Example

FIG. 3 illustrates the effect of five illustrative substituted perhydropyrrolopyridines on HIV replication in IL-2 and phytohaemagglutinin (PHA)-stimulated cultured human peripheral blood mononuclear cells (PBMC) infected with HIV.

Example

FIG. 1 illustrates the effect of Example 1 on HIV replication in IL-2 and phytohaemagglutinin (PHA)-stimulated cul-

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tured human peripheral blood mononuclear cells (PBMC) infected with HIV, as a function of dose. All results represent three independent experiments performed in quadruplicates; total donors (n)=3; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0005$ in comparison with vehicle (1.25 μ M DMSO).

PBMCs were infected for five hours and washed three times with media. PBMCs were then treated with Example 1 at 2 μ M, 10 μ M, and 25 μ M. HIV p24 was measured 7 days post-infection. Example 1 inhibited HIV p24 at 25 μ M. PBMCs were treated with Example 1 at 2 μ M, 10 μ M, and 25 μ M for three days. MTS assay was performed post 3 days of treatment, and readings were taken at 490 nm. Cells were viable even at the highest dosage at 25 μ M.

Example

Cell viability is assayed by the "CellTiter 96® Aqueous One Solution Cell Proliferation Assay" (Promega, #G3582), which assesses the metabolic activity of cells by measuring their ability to reduce MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium in the presence of phenazine methosulfate into a formazan product that has an absorbance maximum at 490-500 nm (4). For this assay, 106 stimulated PBMC in mL of growth medium are each aliquoted into 1.5 mL microcentrifuge tubes and treated with the substituted perhydropyrrolopyridines at a final concentration of 25 μ M. The control is only treated with a final concentration of 0.5% DMSO, which is the substituted perhydropyrrolopyridines solvent. After treatment, 105 PBMC in 100 μ L growth medium are each inoculated into a well of a 96 well flat bottom plate with 4 replicates for the control and each of the substituted perhydropyrrolopyridines. After 6 days of incubation of the plate at 37° C. with 5% CO₂ in a humidified incubator treatment, 20 μ L of MTS solution (Promega, #G3582) is added to each well and the plate incubated for 4 hrs at 37° C. with 5% CO₂ in a humidified incubator, at which time the developing color is read at an absorbance of 490 nm on a plate reader.

Example

FIG. 2 illustrates the effect of five illustrative substituted perhydropyrrolopyridines on the viability of IL-2 and phytohaemagglutinin (PHA)-stimulated cultured human peripheral blood mononuclear cells (PBMC).

Example

HIV entry. For HIV entry analysis, stimulated PBMCs are pre-treated with test compounds at desired concentrations and AZT (control) for 1 h and 4 h. The PBMCs are then infected with HIV-Bal (2 ng/mL per 10⁶ cells) for 5 h. The cells are then washed with media and treated with trypsin to remove bound virus. The cells are washed two more times and RNA from samples isolated using RNeasy MiniKit (Qiagen). DNA contamination is removed by DNaseI (Sigma) treatment at RT for 15 min followed by denaturation of DNaseI at 70° C. for 10 min. cDNA synthesis is performed using qScript cDNA supermix. Using this cDNA, real time RT-PCR is performed to quantify target genes of interest. The following primers are used to amplify HIV transcripts: HIV LTR, Forward 5'-TCAAGTGAGTGCCCGGTT (SEQ ID NO: 1) and Reverse 5'-AGCTCCGGTTTCTCTTTTCGCT (SEQ ID NO: 2) and GAPDH-Forward 5'-TGACTTCAACAGCGACACCACT (SEQ ID NO: 3) and Reverse 5'-AC-

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CACCCTGTTGCTGTAGCCAAAT (SEQ ID NO: 4). GAPDH is used as endogenous control. Example 1 did not have an effect on HIV entry.

Example

HIV reverse transcription assay. Compounds described herein inhibit 2LTR circles. Stimulated PBMCs are infected with HIV-Bal (2 ng/mL per 10⁶ cells) for 5 h. PBMCs are then treated with compounds at desired concentrations and AZT (control) for 72 h post-infection. The cells are then washed three times with media and genomic DNA is prepared from samples using DNeasy Blood and Tissue Kit (Qiagen). For real time PCR analysis, the following primers are used to amplify 2LTR circles (by-products of reverse transcription): Forward 5'-AACTAGGGAACCCACTGCTTAAG (SEQ ID NO: 5) and Reverse 5'-CCCACAAATCAAGGATATCT-TGTC (SEQ ID NO: 6). AZT (1.25 μ M, a reverse transcriptase inhibitor) significantly inhibits HIV reverse transcription. FIG. 4 shows that Example 1 significantly inhibits reverse transcription at each concentration and appears to start to inhibit at the reverse transcription level. All results represent two independent experiments performed; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0005$ in comparison with vehicle (1.25 μ M DMSO).

Example

HIV Genomic DNA Integration Compounds described herein show inhibition at the viral integration. Stimulated PBMCs are infected with HIV-Bal (2 ng/mL per 10⁶ cells) for 5 h. The PBMCs are then treated with compounds at desired concentrations and AZT (control) for 72 h post-infection. The cells are then washed three times with media. Genomic DNA is prepared from samples using DNeasy Blood and Tissue Kit (Qiagen). ALU-PCR is performed followed by real time PCR to quantify integrated viral genome. The following primers are used to amplify HIV transcripts: HIV LTR, Forward 5'-TCAAGTGAGTGCCCGGTT (SEQ ID NO: 1) and Reverse 5'-AGCTCCGGTTTCTCTTTTCGCT (SEQ ID NO: 2) and GAPDH-Forward 5'-TGACTTCAACAGCGACACCACT (SEQ ID NO: 3) and Reverse 5'-ACCACCCTGT-TGCTGTAGCCAAAT (SEQ ID NO: 4). GAPDH is used as endogenous control. AZT (1.25 μ M, a reverse transcriptase inhibitor) significantly inhibits HIV viral integration. FIG. 5 shows that Example 1 significantly inhibits viral integration at each concentration. All results represent two independent experiments performed; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0005$ in comparison with vehicle (1.25 μ M DMSO).

Example

HIV transcription. Compounds inhibit at the viral transcription level. Stimulated PBMCs are infected with HIV-Bal (2 ng/mL per 10⁶ cells) for 4-6 h. PBMCs are then treated with compounds at desired concentrations and AZT (control) for 72 h post-infection. The cells are then washed three times with media and genomic RNA is prepared from samples using RNeasy MiniKit (Qiagen). Real time RT-PCR is performed to detect mature viral early (Rev) and late transcripts (Gag and Env). The following primers are used to amplify HIV transcripts: Rev, Forward 5'-TCCTTGGCACT-TATCTGGGACGAT (SEQ ID NO: 7) and Reverse 5'-TCCAGAAAGTTCCACAATCCTCGT (SEQ ID NO: 8); Env, Forward 5'-ACGAGGATTGTGGAAGTCTGGGA (SEQ ID NO: 9) and Reverse 5'-TGGCATTGAGCAAGCTAACAGCAC (SEQ ID NO: 10); Gag, Forward 5'-AGAGAAG-

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GCTTTCAGCCCAGAAAGT (SEQ ID NO: 11) and Reverse 5'-TGCACTGGATGCACTCTATCCCAT (SEQ ID NO: 12); GAPDH, Forward 5'-TGACTTCAACAGCGACACCCACT (SEQ ID NO: 3) and Reverse 5'-ACCACCCTGTTGCTGTAGCCAAAT (SEQ ID NO: 4). GADPH is used as endogenous control. AZT (1.25 μ M, a reverse transcriptase inhibitor) significantly inhibits HIV transcription. FIG. 6 shows that Example 1 significantly inhibits viral transcription at each concentration. All results represent two independent experiments performed; ***= $p < 0.0005$ in comparison with vehicle (1.25 μ M DMSO).

Example

Assay for Anti-BVDV efficacy. Cells: Bovine Turbinate (BT) cells maintained as monolayers in disposable cell culture labware are used for the antiviral efficacy test. Prior to testing, host cell cultures are seeded onto the 96-well cell culture plates and used approximately 48 hours after seeding. Cells are cultured to achieve monolayers of 80-90% confluence. The growth medium (GM) and maintenance medium (MM) include Dulbecco's Modified Eagle Media (DMEM) with L-glutamine (ATCC #30-2002), 10% Horse serum and penicillin/streptomycin (10,000 units of penicillin and 10,000 μ g of streptomycin per mL, Life Technologies #15140-122 or similar) for a final concentration of 100 units penicillin and 100 μ g streptomycin in the medium.

Example

Bovine Viral Diarrhea Virus strain NADL from BSLI high-titer virus stock is used. Prior to use, aliquots of the stock virus are removed and thawed from a -70° C. freezer. The BVDV is diluted in a maintenance medium (MM) to obtain 0.1 Multiplicity of Infection (MOI).

Example

Cytopathic (CPE) assay. CPE refers to degenerative changes in BT tissue culture induced by BVDV as a consequence of its multiplication. BT cell cultures are washed with PBS, and 100 μ L aliquots of MM are added to the cells and incubated in a CO₂ incubator for 2 hours. After incubation, the MM is removed; the cells washed again with PBS and overlaid with 100 μ L of the different concentrations of the tested compounds. The plates are incubated in a CO₂ incubator for 48 to 72 hours. Upon completion of incubation, the plates are evaluated for test compound-induced inhibition of CPE using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole (MTT) assay. This assay is a colorimetric assay that measures the activity of enzymes that reduce MTT to the purple color formazan dye. CPE is confirmed using an Inverted Compound Microscope. FIG. 7 shows the efficacy of Example 1 on BVDV and the lack of apparent cytotoxicity in BT cells. FIG. 8 shows the efficacy of Example 4 on BVDV and the lack of apparent cytotoxicity in BT cells. FIG. 9 shows the efficacy of Example 10 on BVDV and the lack of apparent cytotoxicity in BT cells. FIG. 10 shows the efficacy of Example 101 on BVDV and the lack of apparent cytotoxicity in BT cells. Illustrative data are shown in the following table.

Example	IC50 (μ M)	95% CI	IC90 (μ M)	95% CI
1	6.2	5.9-6.6	33.5	27.4-40.9
4	7.3	6.9-7.6	17.0	15.5-18.6

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-continued

Example	IC50 (μ M)	95% CI	IC90 (μ M)	95% CI
10	4.9	4.2-5.7	19.6	14.1-27.1
101	15.9	14.0-18.2	564.8	234.7-1359

Prior to the CPE assays, test compounds are tested to determine the highest non-cytotoxic concentration. Cell cultures are washed with PBS, overlaid with 100 μ L of MM and incubated for 2 hours. After incubation, the MM is replaced with 100 μ L aliquots of the test compounds at different concentrations. The cytotoxicity test includes a DMSO control (dose not to exceed 0.5%). The plates are incubated in a CO₂ incubator for 48 to 72 hours. Toxicity is evaluated using the MTT assay. The tests and assays are performed twice in duplicates. Results showing a significant difference are repeated two more times.

Method Example

The following protocol is used for the evaluation of PK parameters and tolerance of the animals for the test compound. The protocol includes three escalating dose levels for each of the compounds administered at a volume of 5 mL/kg once a day by intra-peritoneal (IP) injection. The tolerance is determined over a fourteen day treatment course. The study animals include three 5-mouse groups. Also included is one 5-mouse control group injected with 5 mL/kg of a vehicle. The mouse groups include both male and female 3-month old mice, such as murine KMT Mice™, with a weight range of ≥ 12.0 g. Blood samples are drawn via the central tail artery of the animal for measurement of serum concentrations of the substituted perhydro pyrrolopyridines on the morning of Day 8 immediately prior to the compound dose (trough sample 24 hours post the Day 7 dose) and on the morning of Day 15 (trough sample 24 hours post the final Day 14 dose). A volume of approximately 100 μ L is collected into tubes, allowed to clot at 2-8° C., centrifuged, and the serum removed from above the clot pellet and stored frozen at -80° C. until ready for concentration measurement.

Example

MTS (Cell Viability) assay. Test compounds are also evaluated in a conventional cytotoxicity assay. Uninfected and stimulated PBMCs are plated in a 96 well flat-bottom plate at 200,000 cells in 200 μ L volume of the media in quadruplicates for 3 days at 37° C., in a 5% CO₂, humidified incubator. 20 μ L of CellTiter 96 Aqueous One Solution Reagent (Promega) is added to each well of the plate, and the plate is incubated at 37° C., in a 5% CO₂, humidified incubator for 3 h. The plate is then read on a plate reader at 490 nm. The readings are then measured as percentage of viability relative to the control. In each case, test compound did not exhibit cytotoxicity at 5 μ M. It is appreciated that the lack of cytotoxicity supports the conclusion that the test compound activity in reducing viral titer is specific to the viral disease.

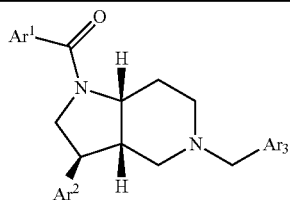
Example

Micro-Ames Test for Mutagenicity. Compounds described herein are not mutagenic. Compounds described herein and tested in this assay did not show mutagenicity at the highest concentrations tested (as high as 500 μ g/plate) in rat liver microsomes.

Example	Ar ¹	Ar ²	Ar ³
15	1	Ph	4-F—C ₆ H ₄
	2	3-Pyridyl	4-F—C ₆ H ₄
	3	Ph	4-F—C ₆ H ₄
	4	Ph	4-MeO—C ₆ H ₄
	5	2-F—C ₆ H ₄	3-Thienyl
	6	Ph	4-F—C ₆ H ₄
20	7	2-Thienyl	4-F—C ₆ H ₄
	8	Ph	4-F—C ₆ H ₄
	9	Ph	4-F—C ₆ H ₄
	10	Ph	4-MeO—C ₆ H ₄
	11	2-F—C ₆ H ₄	3-Thienyl
	12	2-F—C ₆ H ₄	3-Thienyl
25	13	Ph	3-MeO—C ₆ H ₄
	14	3-Pyridyl	4-F—C ₆ H ₄
	15	2-Thienyl	4-F—C ₆ H ₄
	16	2-Thienyl	4-F—C ₆ H ₄
	17	Ph	3-MeO—C ₆ H ₄
	18	Ph	3-MeO—C ₆ H ₄
30	19	2-F—C ₆ H ₄	3-Thienyl
	20	Ph	3-MeO—C ₆ H ₄
	21	Ph	3-MeO—C ₆ H ₄
	22	3-Pyridyl	4-F—C ₆ H ₄
	23	2-Pyridyl	4-F—C ₆ H ₄
	24	3-Pyridyl	4-F—C ₆ H ₄
35	25	Ph	4-MeO—C ₆ H ₄
	26	2-Pyridyl	4-F—C ₆ H ₄
	27	2-Pyridyl	4-F—C ₆ H ₄
	28	Ph	4-MeO—C ₆ H ₄
	29	2-Pyridyl	4-F—C ₆ H ₄
	30	Ph	3-MeO—C ₆ H ₄
40	31	Ph	3-Thienyl
	32	Ph	3-Thienyl
	33	4-Pyridyl	3-Thienyl
	34	4-Pyridyl	3-Thienyl
	35	2-F—C ₆ H ₄	3-Thienyl
	36	2-F—C ₆ H ₄	3-Thienyl
	37	2-F—C ₆ H ₄	3-Thienyl
45	38	2-F—C ₆ H ₄	3-Thienyl
	39	2-F—C ₆ H ₄	3-Thienyl
	40	2-F—C ₆ H ₄	3-Thienyl
	41	2-F—C ₆ H ₄	3-Thienyl
	42	4-F—C ₆ H ₄	3-Thienyl
	43	Ph	3-MeO—C ₆ H ₄
50	44	Ph	3-MeO—C ₆ H ₄
	45	Ph	3-MeO—C ₆ H ₄
	46	Ph	3-MeO—C ₆ H ₄
	47	Ph	3-MeO—C ₆ H ₄
	48	3-Pyridyl	4-F—C ₆ H ₄
	49	3-Pyridyl	4-F—C ₆ H ₄
55	50	2-Pyridyl	4-F—C ₆ H ₄
	51	2-Pyridyl	4-F—C ₆ H ₄
	52	2-Pyridyl	4-F—C ₆ H ₄
	53	2-Pyridyl	4-F—C ₆ H ₄
	54	2-Thienyl	4-F—C ₆ H ₄
	55	2-Thienyl	4-F—C ₆ H ₄
	56	2-Thienyl	4-F—C ₆ H ₄
60	57	2-Thienyl	4-F—C ₆ H ₄
	58	Ph	4-MeO—C ₆ H ₄
	59	Ph	4-MeO—C ₆ H ₄
	60	3-Pyridyl	4-F—C ₆ H ₄
	61	3-Pyridyl	4-F—C ₆ H ₄
	62	3-Pyridyl	4-F—C ₆ H ₄
65	63	3-Pyridyl	4-F—C ₆ H ₄
	64	3-Pyridyl	4-F—C ₆ H ₄

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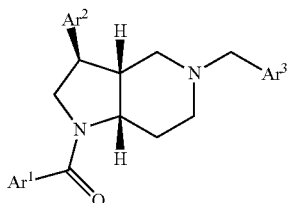
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Example	Ar ¹	Ar ²	Ar ³
65	3-Pyridyl	4-F—C ₆ H ₄	3-Thienyl
66	4-Pyridyl	4-F—C ₆ H ₄	4-MeO—C ₆ H ₄
67	4-Pyridyl	4-F—C ₆ H ₄	2-Thienyl
68	4-Pyridyl	4-F—C ₆ H ₄	3-Thienyl

Compound Example 2

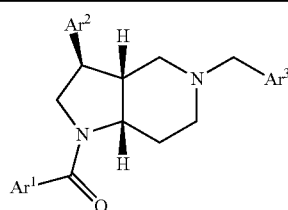
The following compounds are described herein:



Example	Ar ¹	Ar ²	Ar ³
101	Ph	4-MeO—C ₆ H ₄	4-F—C ₆ H ₄
102	4-Pyridyl	4-F—C ₆ H ₄	2-Cl—C ₆ H ₄
103	Ph	3-Thienyl	2-F—C ₆ H ₄
104	Ph	4-F—C ₆ H ₄	3-F—C ₆ H ₄
105	Ph	3-Thienyl	Ph
106	Ph	3-Thienyl	4-F—C ₆ H ₄
107	Ph	3-Thienyl	4-Cl—C ₆ H ₄
108	Ph	3-Thienyl	3,4-F ₂ —C ₆ H ₃
109	3-Pyridyl	4-F—C ₆ H ₄	2-MeO—C ₆ H ₄
110	4-Pyridyl	4-F—C ₆ H ₄	3-Me—C ₆ H ₄
111	Ph	4-F—C ₆ H ₄	1-Me-pyrazol-4-yl
112	Ph	4-F—C ₆ H ₄	1,5-Me ₂ -pyrazol-4-yl
113	Ph	4-F—C ₆ H ₄	1,3-Me ₂ -pyrazol-4-yl
114	Ph	3-Thienyl	4-MeO—C ₆ H ₄
115	4-Pyridyl	3-Thienyl	4-F—C ₆ H ₄
116	4-Pyridyl	3-Thienyl	4-Cl—C ₆ H ₄
117	4-Pyridyl	3-Thienyl	3-Cl—C ₆ H ₄
118	4-Pyridyl	3-Thienyl	3,4-F ₂ —C ₆ H ₃
119	4-F—C ₆ H ₄	3-Thienyl	Ph
120	4-F—C ₆ H ₄	3-Thienyl	4-F—C ₆ H ₄
121	3-Pyridyl	4-F—C ₆ H ₄	3-MeO—C ₆ H ₄
122	4-Pyridyl	4-F—C ₆ H ₄	2-MeO—C ₆ H ₄
123	2-Pyridyl	4-F—C ₆ H ₄	2-Cl—C ₆ H ₄
124	4-Pyridyl	4-F—C ₆ H ₄	4-F—C ₆ H ₄
125	2-Pyridyl	4-F—C ₆ H ₄	4-F—C ₆ H ₄
126	2-Pyridyl	4-F—C ₆ H ₄	3-F—C ₆ H ₄
127	Ph	3-Thienyl	2-Cl—C ₆ H ₄
128	Ph	3-Thienyl	3-Pyridyl
129	Ph	3-Thienyl	3-MeO—C ₆ H ₄
130	Ph	3-Thienyl	2-MeO—C ₆ H ₄
131	Ph	3-Thienyl	3-Cl—C ₆ H ₄
132	Ph	3-Thienyl	2-Me—C ₆ H ₄
133	Ph	3-Thienyl	3-Me—C ₆ H ₄
134	Ph	3-Thienyl	3-F—C ₆ H ₄
135	Ph	3-Thienyl	2,3-F ₂ —C ₆ H ₄
136	4-Pyridyl	3-Thienyl	Ph
137	4-Pyridyl	3-Thienyl	2-F—C ₆ H ₄
138	4-Pyridyl	3-Thienyl	2-Cl—C ₆ H ₄
139	4-Pyridyl	3-Thienyl	4-MeO—C ₆ H ₄
140	4-Pyridyl	3-Thienyl	4-Me—C ₆ H ₄
141	4-Pyridyl	3-Thienyl	4-i-Pr—C ₆ H ₄

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-continued



Example	Ar ¹	Ar ²	Ar ³
142	4-Pyridyl	3-Thienyl	3-MeO—C ₆ H ₄
143	4-Pyridyl	3-Thienyl	2-MeO—C ₆ H ₄
144	4-Pyridyl	3-Thienyl	2-Me—C ₆ H ₄
145	4-Pyridyl	3-Thienyl	2,3-F ₂ —C ₆ H ₄
146	4-F—C ₆ H ₄	3-Thienyl	2-F—C ₆ H ₄
147	4-F—C ₆ H ₄	3-Thienyl	4-Me—C ₆ H ₄
148	4-F—C ₆ H ₄	3-Thienyl	4-Pyridyl
149	4-F—C ₆ H ₄	3-Thienyl	3-Pyridyl
150	4-F—C ₆ H ₄	3-Thienyl	2-Pyridyl
151	4-F—C ₆ H ₄	3-Thienyl	2-Me—C ₆ H ₄
152	4-F—C ₆ H ₄	3-Thienyl	3-Me—C ₆ H ₄
153	4-F—C ₆ H ₄	3-Thienyl	3-F—C ₆ H ₄
154	2-Pyridyl	4-F—C ₆ H ₄	2-Me—C ₆ H ₄
155	3-Pyridyl	4-F—C ₆ H ₄	3-Pyridyl
156	3-Pyridyl	4-F—C ₆ H ₄	2-Pyridyl
157	3-Pyridyl	4-F—C ₆ H ₄	3-F—C ₆ H ₄
158	3-Pyridyl	4-F—C ₆ H ₄	1,5-Me ₂ -pyrazol-4-yl
159	3-Pyridyl	4-F—C ₆ H ₄	1,3-Me ₂ -pyrazol-4-yl
160	4-Pyridyl	4-F—C ₆ H ₄	Ph
161	4-Pyridyl	4-F—C ₆ H ₄	2-F—C ₆ H ₄
162	4-Pyridyl	4-F—C ₆ H ₄	4-Pyridyl
163	4-Pyridyl	4-F—C ₆ H ₄	3-MeO—C ₆ H ₄
164	4-Pyridyl	4-F—C ₆ H ₄	2-Pyridyl
165	4-Pyridyl	4-F—C ₆ H ₄	2-Me—C ₆ H ₄
166	4-Pyridyl	4-F—C ₆ H ₄	3-F—C ₆ H ₄

The following publications, and each additional publication cited herein, are incorporated herein by reference.

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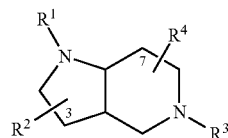
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What is claimed is:

1. A method for treating a host animal with HIV, the method comprising the step of administering to the host animal a pharmaceutical composition comprising a therapeutically effective amount of one or more compounds of the formula



or a pharmaceutically acceptable salt thereof, wherein:

R¹ is arylcarbonyl, wherein aryl is monocyclic and is optionally substituted with halo or alkyl;

R² is aryl, wherein aryl is monocyclic and is optionally substituted with halo, alkyl, or alkoxy, and wherein R² is at C-3;

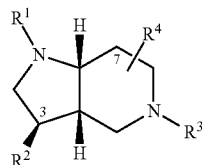
R³ is arylmethyl, wherein aryl is monocyclic and is optionally substituted with halo, alkyl, or alkoxy; and

R⁴ is hydrogen;

wherein the pharmaceutical composition optionally comprises one or more pharmaceutically acceptable carriers, diluent, or excipients, or combinations thereof.

2. The method of claim 1 wherein the stereochemistry of the ring fusion in the compound is syn.

3. The method of claim 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

4. The method of claim 1 wherein R¹ is optionally substituted benzoyl.

5. The method of claim 1 wherein R¹ is benzoyl.

6. The method of claim 1 wherein R¹ is picolinoyl.

7. The method of claim 1 wherein R¹ is 3-picolinoyl.

8. The method of claim 1 wherein R² is optionally substituted phenyl.

9. The method of claim 1 wherein R² is phenyl substituted with halo, alkoxy, or a combination thereof.

10. The method of claim 1 wherein R² is halophenyl.

11. The method of claim 1 wherein R² is alkoxyphenyl.

12. The method of claim 1 wherein R² is methoxyphenyl.

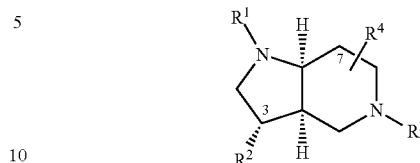
13. The method of claim 1 wherein R³ is optionally substituted benzyl.

14. The method of claim 1 wherein R³ is halo substituted benzyl.

15. The method of claim 1 wherein R³ is fluorobenzyl or chlorobenzyl.

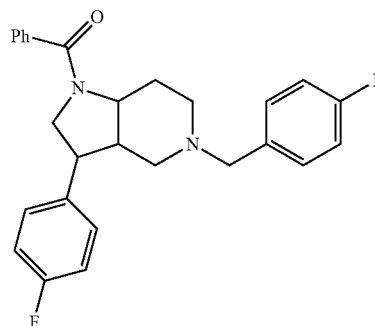
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16. The method of claim 1 wherein the compound is of the formula



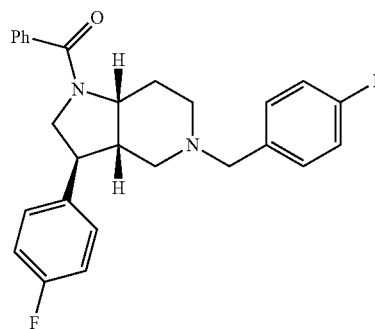
or a pharmaceutically acceptable salt thereof.

17. The method of claim 1 wherein the compound is of the formula



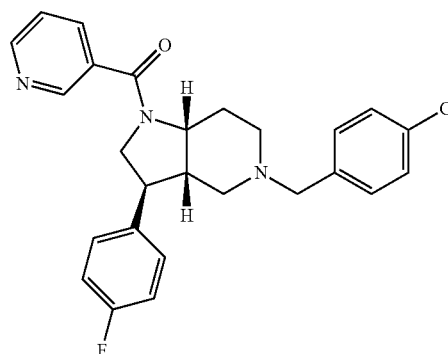
or a pharmaceutically acceptable salt thereof.

18. The method of claim 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

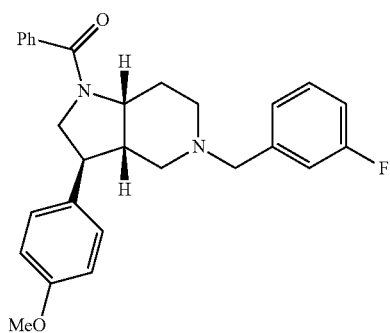
19. The method of claim 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

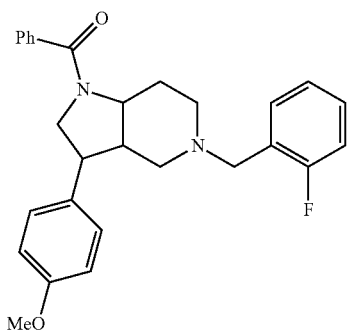
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20. The method of claim 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

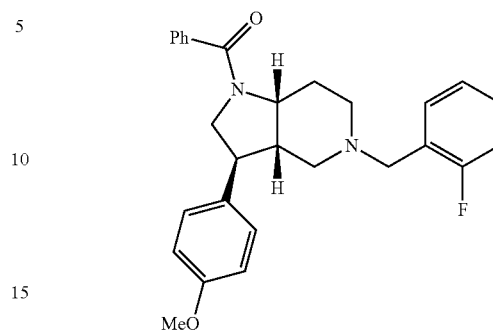
21. The method of claim 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

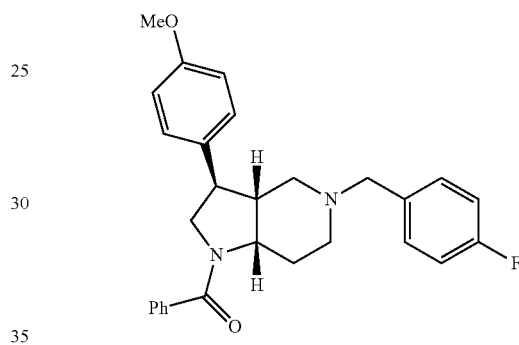
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22. The method of claim 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

23. The method of claim 1 wherein the compound is of the formula



or a pharmaceutically acceptable salt thereof.

24. The method of claim 1 wherein the pharmaceutical composition is administered in combination with a second HIV infection-treating agent.

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